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GRAY MOLD OF CASTOR BEAN [1]

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INTRODUCTION

The castor bean (*Ricinus communis* L.) has had a unique history as a commercial crop in America. From 1860 to 1900 it was a common crop, particularly in Oklahoma, Kansas, Missouri, and Illinois (*7, p. 295*).[3] Climate and soil conditions in some parts are highly suitable for its more general culture, as witnessed by the thousands of dooryard clusters of plants that may be seen throughout the Southern States. Furthermore, there has always been a sufficient home market for the product, the United States consuming annually something like a million bushels (*13*). Nevertheless production had gradually dropped off, and for years previous to the World War, the crop was grown only sporadically in limited areas in the South. The one factor which has prevented the crop from being a success in this country is an economic one, namely, inability to compete with foreign producing sections. The bulk of castor beans used in this country have for years come from India, where producing costs are low.

In one year, and for one year only, this state of affairs was completely changed. In 1918 large areas were devoted to the crop all the way from South Carolina to California, along the southern border. Something like 70,000 acres were planted altogether. The reason was, obviously, the great war, and the purpose, to provide the necessary lubricant, castor oil, for the rotary engines used in a part of the immense new fleet of aeroplanes. The usual source of supply, India, was virtually cut off by the war-emergency need of practically all shipping for other purposes. Consequently it was necessary that the supply be produced in this country. The first need was, of course, for an adequate amount of seed. Sufficient seed did not exist in America. This problem was met eventually through the activities of the War and State Departments, by having a shipload of castor beans from Bombay, India, diverted from England, where it had been destined to be made immediately into oil. The seed was distributed by an organization perfected by the War Department to farmers in the Southern States who agreed to grow them for a guaranteed price per bushel.

Early in the year before this unusual crop was planted a thorough search in the literature was made to determine to what diseases, if any,

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[2] The writer wishes to express his thanks to Mr. H. R. Fulton and Mr. J. R. Winston, of the Office of Fruit-Disease Investigations, for the use of the laboratory facilities at Orlando, Fla., where most of the investigations recorded in this paper were carried on. During two months, January and June, 1919, work on the problem was done at Cornell University, Ithaca, N. Y. The writer wishes to make special acknowledgment to Prof. H. H. Whetzel for the use of his own personal laboratory and greenhouse facilities, and for his invaluable suggestions and help throughout the course of the work.

[3] Reference is made by number (italic) to "Literature cited," p. 714-715.

the crop was subject. Only one was reported as occurring in America, a seedling blight in Mississippi in 1892 (*18*). Apparently but little work had been done on the disease at the time, and as the crop was not continued in later years no further investigations were made. The common dooryard castor bean of the South, "Palma Christi" as it is widely called, has always been so free from disease that it was commonly believed to be immune. In fact, there was a widespread superstition that it had a beneficial effect on other crops and even on chickens, around whose yards it is often planted. It was believed that the castor bean kept both kitchen garden and chickens from attack by insect pests.

Outside of America the most serious disease reported was a Phytophthora seedling disease and leaf blight in India, described by Dastur in 1913 (*6*). A few other minor diseases including a damping-off of seedlings, a rust, and a leaf spot, also occur in India. The diseases on the whole were not abundant and not serious. In this connection Dastur says in his paper (*6*, p. 178):

> Though the castor-oil plant is so widely distributed, still no important fungus pest, except the rust, has been recorded as attacking this crop.

Thus, in so far as plant diseases were concerned, the outlook was good for a successful season. Early in the summer, however, a number of pests appeared and required the attention of both entomologists and plant pathologists. The first alarm was caused by an outbreak of the semitropical army worm *Xylomyges eridania* Cr.[4] It practically stripped large portions of some fields of all green parts, resulting in the death of many of the plants. This was followed by the bacterial wilt, an account of which has been published by Smith and Godfrey (*14*). This, though widespread in its distribution, did not cause a very high percentage of loss. A few other insects made their appearance, particularly the red spider (*Tetranychus quinquenychus* McGregor) and the pink corn worm (*Pyroderces rileyi* Wals.)[4] In addition, two leaf spots made their appearance, one caused by *Cercospora ricinelli* Sacc. and Berl. (*4*) and the other by a bacterium. Neither was very serious. Finally, in July a gray mold appeared upon the inflorescence as the most serious menace of all. Members of the State Plant Board of Florida first reported its occurrence in different parts of the State to their headquarters at Gainesville. Entomologists who were combating the semitropical army worm noticed the increased seriousness of the gray mold as the rainy season advanced. The disease became more widespread and definitely more destructive. Specimens were sent to Washington to the Office of Pathological Collections, and the fungus was found to be a new species of Botrytis. Prof. H. E. Stevens, of the Florida Experiment Station, first published a report of the disease (*16*, *17*). A brief mention of it was also made in a Cuban publication (*4*). Later the writer, who was assigned to the investigation of the problem by the Bureau of Plant Industry, published a description of the causal organism (*10*).

Inasmuch as this castor bean disease was a new one in America, and as its development was gradual during the season, from a few sporadic primary infections to widespread destructiveness, the writer has attempted to treat the different phases of his investigations in so far as practicable in chronologic order, in order to better record its interesting history.

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[4] KISLIUK, Max, Jr. INSECTS AFFECTING THE CASTOR OIL PLANT. (Manuscript in Bur. Ent., U.S. Dept. Agr.)

RANGE OF THE DISEASE

A preliminary survey was made early in August, 1918. The disease was found to be especially serious in Florida, which State was more extensively planted to the crop than any other. At Gainesville it was well scattered over a large field. At Orlando it had just made its appearance on a small scale. At Tampa, Seffner, and Fort Meade, it had already destroyed a considerable percentage of the blossoms and young pods and was rapidly spreading. The most serious outbreak of the trouble was at De Land, on the east coast. Here on August 10, approximately 50 per cent of the inflorescences were affected, and continuous moist weather gave promise of still further development of the disease. Farther south along the east coast, at Vero, West Palm Beach, Miami, and Davie, the disease had not yet made its appearance. In South Carolina, Alabama, and Georgia it was not to be found. In Louisiana, the first large field examined disclosed none of the fungus. In another field it was found in

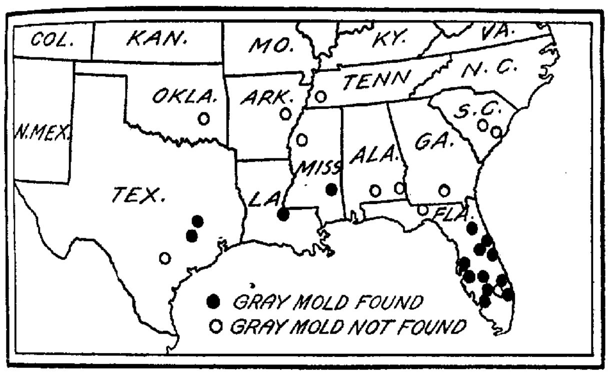

FIG. 1.—Map of the southern portion of the United States, showing the distribution of the gray mold of the castor bean.

abundance. The significance of this diversity became evident when it was learned that the first field was planted to seed grown in America the year before. The second field was planted to the usual Bombay seed. This feature will be emphasized later (p. 701) in connection with the origin of the pathogene. In southern Mississippi the disease was found in one field, in a locality that had been subject to recent heavy precipitation. It was reported by other observers who had become familiar with it to be absent from Texas, Arkansas, Tennessee, and northern Mississippi.

In September and October further surveys were made, to follow up the previous observations and to note any further spread. It was found to have become definitely established in fields at Kissimmee, Lucerne Park, Haines City, and at Moorehaven, on Lake Okeechobee, where a number of fields were seriously affected. In October a report of its occurrence in eastern Texas, following a protracted wet period, was followed up; and it was found that the disease was causing from 10 to 25 per cent loss in fields near Houston and Alvin. At a point in southern Texas not visited by the writer personally the trouble was not present, according to reliable reports. Here the whole summer was known to

be very dry. The absence of the disease in northern Louisiana, Arkansas, Tennessee, and northern Mississippi was further verified at this time by a personal survey. A field at Montgomery, Ala., was also found to be free from the disease. Neither had it occurred since the first visit, in plantings near Valdosta, Ga., Dothan, Ala., or Florence or McBee, S. C.

By November 15 the mold had become exceedingly destructive in localities where it had been seen earlier. At De Land, Fla., where it had been causing perhaps 50 per cent loss in August, it was now affecting practically every inflorescence shortly after it came out of the sheath, making the loss a full 100 per cent. It had also appeared at points along the east coast, particularly near Miami, and it was even found at the plantation at Davie, on the border of the Everglades. Here also it was found to have spread to scattered plants of "Palma Christi."

Figure 1 is a map showing the location of castor bean plantings in the South where the disease did and did not occur as determined by personal field inspections by the writer and reports from others. The losses in those localities where the disease was present varied from 10 to 100 per cent.

INFLUENCE OF WEATHER CONDITIONS ON THE PROGRESS OF THE DISEASE

During the course of the various survey trips through castor-bean growing sections, the writer noted the definite effects of moisture and temperature on the development of the gray mold. Its presence and rapid spread in the various localities in Florida during July, August, and September were definitely traceable to the warm moist weather of those sections, where the wet season regularly occurs in the summer time. In certain localities in particular it was observed that there were showers every day, frequently even several times a day. Plate 2, A, shows typical cloud effects that result in the showery weather referred to. At night there almost invariably occurred a heavy dew. Figure 2 is a copy of a figure in a report of the Weather Bureau (*19*) showing the average monthly rainfall in different sections of Florida. This excessive moisture made an ideal condition for the development of such a fungus as this one, a gray mold.

Figure 3, showing the daily rainfall (*20*) in particular localities for July, August, September, and October presents graphically the striking contrast in rainfall between typical localities where the disease was serious and those in which it did not occur.

Particular attention is called to the correlation between the summer rainfall and the occurrence of castor-bean gray mold. At Bartow, Fla., protracted wet periods occurred throughout the summer, and the disease appeared early and was continuously destructive. At Miami, Fla., except for one period early in July, at which time the plants were not yet fully developed, the wet weather did not begin until September 9, after which there was hardly a day without rain until after the middle of October. There the disease did not begin to be destructive until late summer. Again, at Houston, Tex., continuous heavy rain did not begin till about October 7, and there too, the disease was not reported until late in the season. At Valdosta, Ga., and Florence, S. C., where the fungus did not develop at all, the rainfall that did occur was of short duration and always followed by long periods of dry weather.

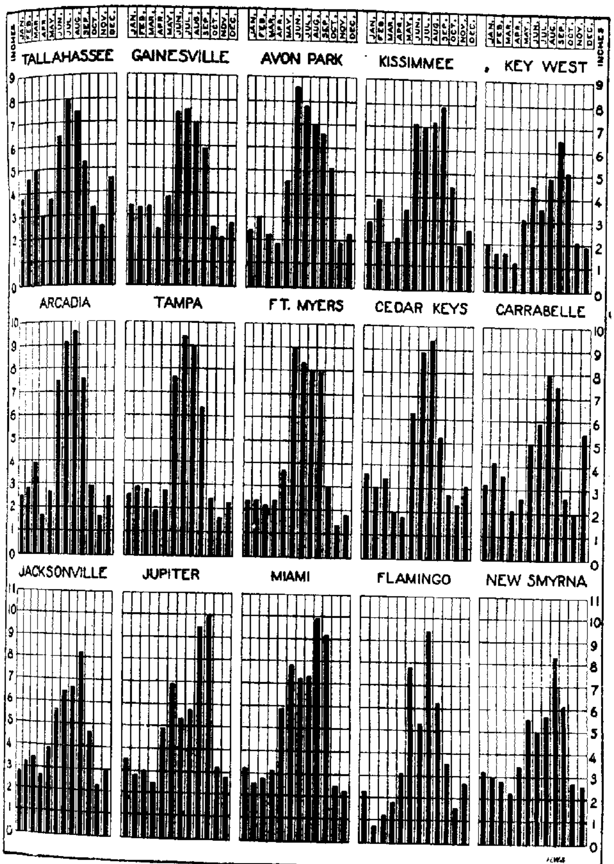

FIG. 2.—Diagrams showing the comparative monthly distribution of precipitation in different parts of Florida, the averages of several years' records.

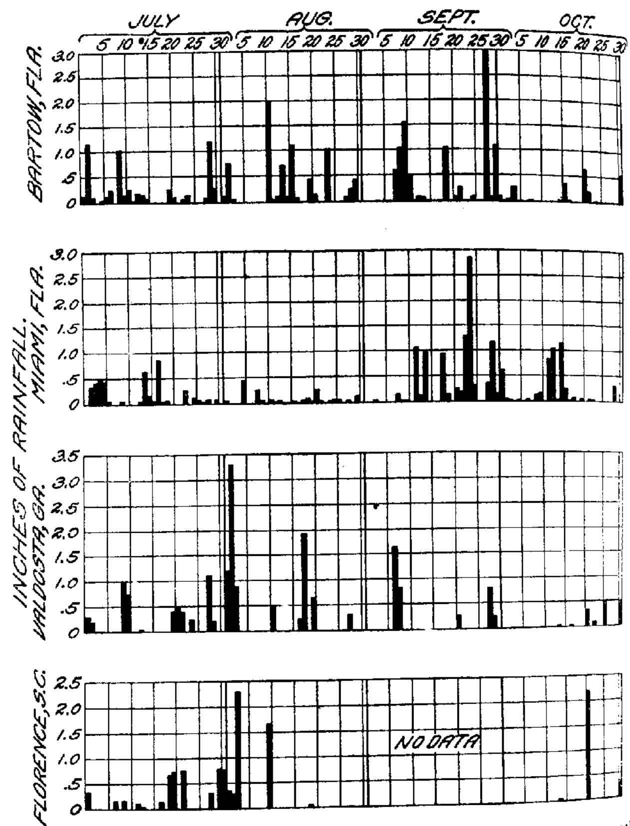

FIG. 3.—Daily precipitation records during the summer season, 1918, showing the difference in rainfall distribution between typical localities where the gray mold occurred (upper two) and where it did not occur (lower two).

The influence of temperature was not so definitely traced, though the indications are that it has its effect as well. As will be seen elsewhere in the paper, the optimum temperature for the development of the fungus in pure culture is approximately 25° C. (77° F.). Where temperature much below this prevailed in a locality otherwise favorable to infection it might be assumed that its failure to appear was due to too low temperature. Such a condition existed at Little Rock, Ark. After October 17, the moisture was favorable but the maximum temperature was above 72° F. on only two days, October 19 and 20, and it was 70° F. or lower practically all the rest of the month. The minimum fell below 60° F. several days. Likewise in Florida, with the cooler weather in the fall, the fungus definitely ceased to progress. It is likely that the fungus would not be nearly so active in a climate a few degrees cooler than Florida, even though moisture conditions were equally favorable.

DESCRIPTION OF THE DISEASE

Professor Stevens has given a very good description of the gray-mold disease in his Florida publications (*16*,*17*). A more complete description might well be given, however, especially of the earlier stages. Infection first becomes evident on leaves, panicles, or stems, in the form of small bluish spots, from which yellowish drops of liquid exude. Shortly after—in the course of only a few hours if weather conditions remain favorable—strands of hyphae may be seen extending from original infected spots along the surface of the host like a spider's web. These strands produce new points of infection. Development is rapid. The fungus infects constantly increasing areas on the host, meanwhile sending forth its fruiting hyphae. The inflorescence or (to use the common, though botanically incorrect terminology) "spike" becomes covered with this at first gray, then olive-drab mold. Plate 1 shows a typical young spike in an early stage of infection. Plate 3 shows three spikes with the mold farther advanced. At this stage the slightest jar will release a veritable cloud of conidia, which spread further destruction to the rest of the field. As an affected inflorescence becomes older, the mass becomes darker in color and more compact, and the stem droops and gradually loses its immature pods or blossoms. (Pl. 4, A, B.) Close examination of an infected spike will always disclose the presence of the mold itself, or some other characteristic feature of the disease to distinguish it from the natural drying of the male blossoms with which it is sometimes confused. The disease is sometimes still further complicated by the presence of the pink corn worm (*Pyroderces rileyi* Wals.), which is said to do injury at this stage.[5] The two conditions are often found together. In fact, specimens sent to Washington for examination were at first misleading because of the constant presence of this worm.

On the leaves, the disease is usually traceable to a small bit of an inflorescence that has fallen from above. From this central point the fungus spreads, producing a dead spot on the leaf, accompanied by the gray mold. Infection may also occur at the margin of a leaf, causing its death in characteristic Botrytis fashion. However, leaf infections are comparatively rare. Infections on the stalk and branches do not readily occur, the tissues here being more mature and consequently more

[5] KISLIUK, Max, Jr. OP. CIT.

resistant. Cases were seen, however, in which actual contact with a diseased inflorescence produced a distinct canker covered with the typical gray mold. In one case observed such a spot completely girdled the stem, killing the parts above, including several flowering branches and leaves. Plate 4 C, shows a canker on a stalk.

Unfortunately for the production of a crop of castor beans, the flowering parts of the plants are the most readily attacked by the fungus. A spike in any stage of development may become infected. If an abundance of the pathogene is present in the field and weather conditions are favorable, every spike will often become infected early in its development, even before the male blossoms have opened. More often, perhaps, infection takes place through the male blossoms. The anthers, water soaked as they often are after one of the summer rains or after a heavy dew, catch the spores of the fungus and permit them to germinate immediately and produce infection. Nearly mature spikes, containing only green pods, may become infected and fall over, maturing only a part or none at all of the beans. Usually a plant showed spikes in all stages, from buds just out of the sheath to nearly mature pods, all alike with evidences of recent infection.

The castor bean is characterized by its possession of extrafloral nectaries. These may occur at various places on the plant, often at the bases of leaf petioles, often on the leaf itself near the point of attachment of the petioles; but they are more numerous among the flowers. These nectaries exude nectar in large drops, which may be seen early in the morning before they have dried off. It was suspected that this nectar might favor infection by the fungus. Beneficial effect of nutrient material in the infection drop to infection by Botrytis has been mentioned by other writers (*3*) in connection with *Botrytis cinerea* Pers. Repeated examinations, however, failed to show that any large proportion of infections occurred in contact with these nectaries. The part played by the nectaries, however, is not small. They attract myriads of insects, principally honey bees, wasps, bumblebees, and ants which may be seen crawling through the spikes, infected and healthy alike, especially after a shower when conditions are ideal for infection. These insects undoubtedly play a large part in carrying infection. Whetzel (*22*) has noted such an insect relationship in connection with the spread of *Botrytis paeoniae* Oud.

The appearance of a badly infected field in the height of the growing season is indeed discouraging in so far as crop prospects are concerned. Plate 2 B shows a castor bean plant in which practically every inflorescence is affected. After the fruiting of the plant is thus arrested, new spurs shoot out with the brave intention of forming new blossoms. But almost invariably these new inflorescences which form throughout the summer sooner or later become infected.

As the growing season draws to a close with the advent of cooler weather in the fall, the plant gradually ceases to make new growth and with freezing weather becomes greatly weakened or dead. After the leaves are shed, the remains of the diseased spikes, with a suggestion of the still recognizable mold, may be seen (Pl. 5, A). Under the conditions of central Florida some life remained in many of the plants, and early in the spring with the gradually rising temperature, small, weak green spurs could be seen springing forth from various parts of the plants. Even earlier than this, however, evidences appeared of the overwintering of the fungus. On February 1, an inspection of the old plants standing in a

diseased field disclosed the first appearance of sclerotia under natural conditions. These were found upon the diseased spikes usually at their bases upon wood that still contained some sap. Plate 1 and Plate 5 A, show some typical examples of this condition. From this time on sclerotia could be found in increasingly greater abundance.

Before the middle of the month there were found sclerotia lower down on the stalk of the plant. It was soon obvious that spurs that had become infected during the first growing season were instrumental in carrying the infection into the cambium layer of the stalk. Here the fungus overwintered. With the advent of growing conditions, coupled with the decreased resistance of the host plant, it started again into active growth, which was semisaprophytic, in all probability, rather than purely parasitic. Sometimes a distinct canker could be seen, with a molded spur in the middle (Pl. 5, B, C). Sclerotia soon became evident, growing up through the cortex, sometimes evident on the surface only as a hump (Pl. 6, C), and again practically formed on the surface. Plate 6, A and B, are standing plants with an abundance of surface sclerotia. For the next two or three months stalk-borne sclerotia were to be found more and more. Many newly formed cankers with subsequent sclerotia were traceable directly to spring infections of new spurs that had started from buds on the stalks.

In early summer the coffee-bean weevil, *Araecerus fasciculatus* De G., listed among the corn-destroying weevils of the South by Cotton (5), was frequently seen on castor-bean plants of the previous season's growth, and the interesting observation was made that it was actually eating the sclerotia of *Sclerotinia ricini* Godf. Several of the weevils were caught and imprisoned and furnished with sclerotia and corn as food. They appeared actually to prefer the sclerotia to the corn. No significance is attached to their liking for this fungus as food, however, since they scarcely made an impression on the sclerotia that were available.

CAUSAL ORGANISM

ISOLATION

The organism was very easy to isolate. Any portion of a diseased inflorescence placed upon a poured plate of potato or corn meal agar at ordinary temperatures would develop the fungus rapidly, and as a rule it would outgrow all contaminating fungi or bacteria, and pure culture transfers could be readily made. In order to obtain definite strains of the fungus, however, the writer followed the precaution of obtaining single spore cultures in practically every case. This was done by lifting in a pair of forceps a small tuft of the fruiting fungus from the diseased material, holding it at the edge of the poured plate and giving a quick blow with the breath, thus blowing spores upon the surface of the plate. The method was suggested by Prof. H. H. Whetzel, who uses it largely in his work with Botrytis. Clear corn-meal agar was used by the writer. Inside of 24 hours well-isolated single spores, already germinating, were picked out and transferred to tubes. In case any particular plate was too thickly sown with spores, or an absolutely sure single spore isolation was in any other way doubtful, transfers were made to another plate of clear agar and the growth of the colony was followed to make sure of its purity.

In this way about 50 original isolations of the fungus were made. The sources and dates and particular information concerning them are listed in Table I.

TABLE I.—*Sources of cultures of Sclerotinia ricini*

Culture (B) No.	Date collected.	Isolation method.	Place collected.	Remarks.
	1918.			
1	Aug. 17	Tissue fragment	De Land, Fla	Isolated in Washington by Ruth F. Allen.
2	Aug. 30	do	do	
3	Oct. 16	Single spore	Orlando, Fla	Young inflorescence.
4	do	do	do	Do.
5	do	do	do	Large young inflorescence bud just out of sheath.
6	do	do	do	
7	do	do	do	An old infection.
8	Oct. 21	do	Haines City, Fla	
9	do	do	Lucerne Park, Fla	
10	do	do	Winter Haven, Fla	
11	do	do	do	A half-mature inflorescence.
12	Oct. 23	do	Moore Haven, Fla	
13	do	do	do	
14	do	do	do	
15	Oct. 29	do	Houston, Tex	Sheldon district.
16	do	do	do	Do.
17	do	do	do	Do.
18	do	Sclerotium	do	Isolated Dec. 5 from sclerotium in above material.
19	Oct. 30	Single spore	Alvin, Tex	
20	do	do	do	
21	Nov. 25	Tissue fragment	Orlando, Fla	From male blossoms.
22	do	do	do	From inflorescence.
23	do	do	Tampa, Fla	Do.
24	Nov. 27	Single spore	Sanford, Fla	From "native" castor beans.
25	Dec. 9	do	Miami, Fla	
26	do	do	do	
27	Dec. 10	do	do	From typical inflorescence.
28	do	do	do	From fallen pod.
29	do	do	Miami, Fla., Larkin (Fla.) district.	Do.
30	do	do	Allapattah, Fla	
31	Dec. 15	do	do	
32	do	do	do	
33	do	do	Miami, Fla., Buena Vista (Fla.) district.	
34	do	do	Miami, Fla. (9 miles north).	
35	do	do	Davie, Fla	From Bombay bean.
36	do	do	do	From "native" bean.
37	Oct. 30	Sclerotium	Alvin, Tex	Isolated Dec. 23.
	1919.			
38	Feb. 7	Single spore	Orlando, Fla	Winter fruiting of the fungus.
39	do	Sclerotium	do	Collected in the field.
40	Apr. 4	Tissue fragment	do	From lesions on spring-infected plants.

TABLE I.—*Sources of cultures of Sclerotinia ricini*—Continued

Culture No.	Date collected.	Isolation method.	Place collected.	Remarks.
	1919.			
41	Mar. 16	Not known	Santiago de las Vegas, Cuba.	Received from S. C. Bruner, Pathologist.
42	..do	....do	....do	Do.
43	May 1	Single ascospore	Orlando, Fla	From apothecia on field sclerotia.
44	May 29	Tissue fragment	....do	Reisolation from plant inoculated with ascospore culture.
45	Dec. 15	....do	Miami, Fla	From seed of lot 33.
	1918.			
46	Oct. 24	....do	Plant City, Fla	Isolated May 27 from light-weight seed from hulling machine.

All these cultures were identical, showing conclusively the association of this one fungus with the castor-bean blight. Most of them, together with bits of the material from which they were isolated, were sent to Prof. Whetzel, at Cornell University, who verified their identity.

APPEARANCE IN CULTURE

Growth of the fungus on artificial media is very rapid. It is spreading and superficial at first, presenting a characteristically glistening appearance on the surface of the medium. Within 48 hours aerial hyphae begin to develop, in the form of erect single strands, the conidiophores. These develop very thickly and produce conidia often as early as the second day of growth. Soon the culture attains a light-gray color from the production of conidia, and as these become more abundant, it gradually becomes darker till it is drab or even dark-olive gray.[6] Figures A and B of Plate 10 are photographs showing the appearance of the fungus on corn meal agar three days after inoculation. About the fourth day sclerotia begin to form at the edges of the culture in a tube. These are anywhere from ½ mm. to 3 or 4 mm. in length, at first pale smoke gray[5] then gradually darker in color till they are black. At first flat and closely appressed to the surface, they gradually become wrinkled and distorted. On a highly nutrient medium, such as oatmeal agar, they are very abundant; on a less nutrient medium, such as one made with a small quantity of corn meal, less so. Appressoria, while present, are usually too small to be seen distinctly with the naked eye. Occasional cultures appeared in which they were massed together enough to produce the characteristic appearance of appressoria in a culture tube. (Pl. 11, A.) The writer's paper containing the description of the fungus (10) contains a drawing of a typical microappressorium.

The general appearance of the fungus in culture is that of a typical Botrytis with the usual abundance of gray mold and black sclerotia.

[6] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D.C. 1912.

MICROSCOPIC APPEARANCE

The development of a culture of this organism is comparatively rapid. The conidia germinate almost immediately on the surface of a poured plate by the development of a germ tube about half the diameter of the spore itself (Pl. 12, B). A cross wall is formed after the tube has grown in length to three or four times the diameter of the conidium, and profuse branching occurs immediately afterward, together with continued growth in all directions from the conidium. The mycelium of this fungus is easily distinguished from that of other fungi commonly found as contaminations in a plate by its large diameter and its general robust appearance. Its contents are distinctly granular and vacuoles are rare except in old cultures. Anastomosing of hyphae is frequent. It may occur between two hyphae which have arisen from the same spore or between two adjacent hyphae from different spores. Plate 13, I and J, are camera-lucida drawings showing anastomosis. The conidiophores may be distinguished in a young culture very early in their development. They arise perpendicularly from a cell of a horizontally growing vegetative hypha, becoming larger and olivaceous in color a short distance above the surface. Plate 12, D, is a drawing of the base of a conidiophore, showing its attachment to the vegetative hypha. Sometimes a single cluster of conidia is produced on the end of such a conidiophore. More frequently, however, the tip will divide and two equal branches will continue the growth. Often these will divide again, and a third or even a fourth division may occur, always dichotomous, the internodes becoming shorter by about half with each subsequent division. Finally the conidial head starts to form, this too by continued dichotomous divisions of the tip (Pl. 12, A). It was difficult at first for the writer to determine clearly the definite mode of attachment of the conidia to the ultimate branch tips of the conidiophore. Finally, by studying the development of very young cultures, this point was made clear. The conidia are borne on very fine attenuations of the ultimate tips of the conidiophore, these final cells being very turgid though thin-walled and fragile. No wall was discernible. Plate 12, C, depicts immature conidia attached to the conidiophore. Upon the maturity of the conidia, or upon the forcible detachment of nearly mature conidia, the ultimate cells of the conidiophore collapse and hang in a shapeless mass on the end of the next branch back, this tip becoming rounded out meanwhile, owing to the turgidity of the cell. It was impossible to see on a mature fruiting branch the exact connection of a mature conidium, owing to the density of the cluster of conidia.

Microconidia are formed in abundance, beginning after the cultures are about 10 days old. The writer never observed them in nature. Media favorable to heavy conidium production, such as corn meal or potato agar, sometimes do not readily produce microconidia. Upon media which do not produce conidia abundantly, such as oatmeal agar, cooked potato plug, and boiled rice, the microspores may often more readily be found. The writer has observed that they may often be found upon the thin piece of medium which has carried the transfer from one plate or tube to another. Microconidia are extremely small, from 2 to 3μ in diameter. The manner of production was readily observed, however, by examining the exposed surface of a poured plate, with 0.5-mm. water immersion objective. Considerable variation was noticeable in the type of conidiophore on which they were borne. Perhaps the

most frequent form was that pictured in Plate 12, F, a reproduction from a camera-lucida drawing. This is a special branch arising from the sterile hypha imbedded in the substratum. In another type the cluster of conidiophores arises directly on a cell of the sterile hypha (*10, Pl. XLI, e*). In still another form a long, slender branch bears upon its tip either a single conidiophore or a group of them, with the microconidia on their tips. Microconidia were observed to best advantage on germinating ascospores, as shown in Plate 12, G, and photomicrographically in Plate 10, C. From one or two to more than a dozen microconidia may develop in succession on the end of a single conidiophore, those that are mature dropping off and forming a distinct group. In general, the microconidia are very similar in all respects to those described by Brierly (*2*) for *Botrytis cinerea*. No germination was seen, however, though looked for diligently. No endoconidia were observed.

It was not deemed profitable to grow this organism on a long series of culture media, since its growth was almost equally vigorous on most media commonly used in the laboratory. There were a few specific differences evidenced that should be recorded. On corn-meal agar heavy production of the gray mold occurred, with comparatively little development of white mycelium and only occasional sclerotia. On potato agar, also, sclerotia were not abundant, but more mycelium was evident. Oatmeal agar at first produced a profusion of conidia, but later this appearance was hidden by more or less of the white mycelium and a great abundance of sclerotia. This was the medium most commonly employed for the development of sclerotia. Boiled rice and potato plugs had fewer conidia, but sclerotia were abundant. Beyerinck's agar produced a thin growth of conidia and few sclerotia. Sterilized sweet clover stems produced large sclerotia and few conidia. The interesting fact was noted that some media at times produced a heavy mat of conidiophores and conidia but no sclerotia at all. Transfers were made from such tubes to oatmeal agar to determine whether this was a character peculiar to the particular strain of the fungus. In every case a normal heavy development of sclerotia was produced on the new medium.

The cardinal temperatures for the growth of the gray mold on corn-meal agar are as follows: Minimum, 12° C.; optimum, 25° C.; maximum, 35° C.

DEVELOPMENT OF THE PERFECT STAGE

PRELIMINARY EXPERIMENTS

Before there had been any intimation of the existence of a perfect stage of this organism, attempts were made to induce sclerotia to fruit or otherwise function.

EXPERIMENT I.—On February 6, at Orlando, Fla., three Petri dishes of moist sand were sterilized and sclerotia from cultures of strains B 35 and B 37 placed thereon, and the whole placed in an incubator at 27° C. Observations were made for several days without noting any developments other than the production of gray mold from the medium at the edge, that had been carried over with the sclerotia. The plates became dry, and finally on March 16 sterile water was added and the plates were further treated as follows: No. 1, in the ice box for 24 hours, then under a glass moist chamber out of doors under fluctuating but on the whole fairly warm temperature conditions. No. 2, submitted to a −10° C. temperature in a freezing mixture for a few hours, the temperature gradually rising to +10° C., after which it was placed out of doors along with No. 1. No. 3, was placed directly under the out of door conditions.

Observations made frequently thereafter failed to show any signs of the development of a perfect stage.

EXPERIMENT II.—February 22. Sclerotia collected from castor-bean plants under natural conditions were sterilized for two minutes in 1 to 1,000 mercuric chlorid, washed in sterile water three changes, and placed partly on plates of agar, partly on plates of moist sterilized sand. The agar plates developed a typical culture of the fungus (B 39, isolation record, p. 688). On the sand, the mature sclerotia produced an abundance of normal conidiophores and conidia.

FIRST APPEARANCE OF APOTHECIA

The first appearance of the perfect stage of this organism was more or less unexpected; consequently it might be of interest to record its history in detail. On one of the cultures sent to Prof. Whetzel at Cornell University, No. B21, collected November 25, 1918, he noticed an apothecial stalk arising from one of the sclerotia. Prof. Whetzel immediately notified the writer at his temporary station, Orlando, Fla., and he found (Mar. 8) in his culture of the same strain (B 21) and also in one other strain (B 1), a similar condition. The cultures had remained on the shelf and had not been examined for some time. These cultures were then watched with jealous care at both places. In the culture at Cornell the stalk developed a little more, then shriveled and dried up. Under what were undoubtedly the more favorable conditions at Orlando, Fla., the culture continued to develop until the cup opened out and reached maturity. Thus there was secured a perfect specimen of the apothecial stage of this fungus which until then had appeared to be merely a new species of Botrytis.

Over anxiety to permit this specimen to reach full maturity resulted in its becoming slightly overmature, as was evidenced by the development of a mealy condition of the disk. Other apothecia were meanwhile rapidly developing, however, and it was clear that working material was to be had in sufficient abundance for immediate needs. Extraordinary precautions were taken with this culture tube to obtain absolutely authentic single ascospore cultures, for it was not known at this time whether or not further specimens could readily be obtained. From the first specimen to mature, mounts were made and drawings and measurements secured. The second was used to start cultures, but these were discarded in favor of the third, a more perfect specimen, from which more clearly reliable results were secured. This apothecium, taken in situ, is shown in Plate 11 A. For the sake of subsequent references it will be designated apothecium C. Its maturity was indicated on March 27 when a cloudiness different from that due to moisture was observed on the inside of the tube near the disk of the apothecium. A scraping was made from this deposit with a sterile platinum needle and examination showed it to consist of long fusoid spores, obviously ascospores that had been ejected from the mature apothecium.

In preparation for the actual work of securing single ascospore cultures, several Van Tieghem cells, slides, and thin cover glasses were sterilized in the hot air oven, within Petri dishes. In addition, tubes of sterile water and sterile vaseline were made ready. Then some of the cover glasses were covered within a small circular area on one side with a thin, smooth film of clear corn meal agar, applied directly from a tube of melted agar with a platinum loop. Apothecium C was then removed from the tube and placed in a sterile Petri dish.

Isolations were made from ascospores derived in three different ways, as follows: (a) Spores ejected directly from the apothecium to an agar-

coated cover slip, ejection being stimulated for the moment by a slight touch on the edge of the desk; (b) a segment cut from the side of the disk and crushed in a drop of sterile water, the resulting suspension of spores being then applied directly to the agar surface of a cover slip with a platinum loop; (c) spores scraped from the side of the tube to which they had been shot when the apothecium was first mature; these were applied as in (b).

In each case, the cover glass was then placed in position over a Van Tieghem cell which had previously been made ready by ringing above and below with sterile vaseline, sealing to a sterile slide, and pouring in a few drops of sterile water. A sterile moist chamber was thus secured which could be moved about as much as desired without fear of contaminating the contents. Several ascospores were observed, singly and in groups, and their germination and development were watched with the higher powers of the microscope. All were practically in one plane so that it was easy to mark with India ink the position of germinating single spores. After a few hours several such single ascospore strains were cut out and removed to a second agar-coated coverglass and cell, where their purity was verified and further development watched. The transfer was accomplished by lifting the coverglass between the thumb and forefinger of the left hand and cutting out the area marked, a millimeter or so square, with a No. 24 platinum-wire needle which had a very thin sharp, knife-like point. Later these single spore strains were transferred to tubes of agar.

Inasmuch as this method of isolating single spores is not used as widely as its practicability would justify, the writer wishes to call attention to it as a simple, quick, and reliable means of obtaining single spores apart from one another and from all contaminations, especially useful when the spores are exceedingly small. It was developed and used by the writer at Iowa State College in 1916–17.[7] Later Durrell (*8*) used the same method for making single spore isolations. Many workers have used the Van Tieghem cell for isolating single spores of fungi, as, for example, Sherbakoff in his work on the genus Fusarium (*12, p. 102–104*), in which he emphasizes the importance of obtaining single spore strains and the difficulties often attendant thereupon; and most noteworthy of all, Emil Christopher Hansen who as long ago as the middle of the nineteenth century used a method very similar to the one here described for securing single yeast cells in fermentation technic (*11, p. 106, 107*). But insofar as the present writer has been able to determine, precisely this method has not previously been described. When spores are large enough to be seen with the low power through the bottom of a Petri dish and contaminations are not a serious factor, this method would not be justified, of course.

The several cultures derived from all of these three sources of ascospores were typical in every way of the Botrytis cultures obtained from conidia. The typical gray mold developed quickly, and this was followed shortly after by sclerotia. Inoculations of castor bean plants made with these cultures, after the usual method (described on p. 703), produced the typical disease. Reisolations made from such diseased plants were held and used, along with numerous other cultures, for later production of apothecia.

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[7] GODFREY, G. H. CULTURAL STUDIES OF LEPTOSPHAERIA CONIOTHYRIUM CAUSING RASPBERRY CANE BLIGHT. Thesis, Iowa State College, 1917. (Not published.)

DEVELOPMENT OF APOTHECIA UNDER ARTIFICIAL CONDITIONS

Immediately upon receiving the intimation of the existence of a perfect stage of this organism the writer took steps to work out a means of producing apothecia under controlled conditions. In view of the fact that the only apothecia found at first were from tissue transfer cultures (isolation record, p. 688, B 1 and B 21), it was thought that the fungus might be heterothallic. Consequently several cultures were made consisting of mixtures of pure-line strains in various combinations of single-conidium, single-ascospore, and tissue-fragment cultures. No differences were noted in the development of sclerotia; and, as soon became evident, there were no differences in apothecium production. A number of other experiments were started, consisting principally of subjecting sclerotia to different conditions with the idea of determining their effect on apothecium production. An account of these experiments follows.

EXPERIMENT III.—March 12. Some sclerotia from the field, one of them large, widespread, and flat, were placed on a layer of moist sand on a glass plate, and the whole was covered with a battery jar. This was placed on the laboratory table near a window. It was watched carefully for several days without developments, then, in the stress of other work, was neglected for some time. During this period the sand became quite dry. Notwithstanding this, on April 16 five apothecial stalks were seen to have risen from the large flat sclerotium. One was about 1 cm. long and 1½ mm. wide at the end, all the others being not so fully developed. The sand was at once moistened before the apothecia had suffered materially. These will be designated lot III A. At the same time additional sclerotia, which we shall call lot III B, were incubated as follows:

Sclerotia from the field.
Sclerotia from strain B 21 B (1).
Sclerotia from strain B 21 C (a) 1, 4, and 7.

These sclerotia were placed on moist sand in well-marked positions and covered with a battery jar, under laboratory conditions.

Observations were recorded as follows:

Lot III A. April 24. The largest apothecium, already noted, is developing very irregularly. The second has a perfect disk 1½ mm. in diam. The third, half as large, has a hollow end. The fourth is somewhat shriveled, and the fifth is growing.

April 26. The first apothecium in its irregular growth is developing two smaller distinct disks on the side of the first.

April 29. Early in the morning the bell jar was lifted, and the second apothecium was observed to shoot forth ascospores in a distinct cloud. April 30, the bell jar was lifted again, and the spores when ejected were caught on a plate of clear corn-meal agar. Single ascospore isolations were made. Apothecia I and II of this lot were photographed and later preserved in alcohol. The third was sent to Prof. Whetzel at Cornell University.

May 9. A dozen or so more apothecia, well developed, were noted from this lot.

Lot III B. May 6. Apothecial stalks are developing from both field and pure culture sclerotia.

May 10. One or two apothecia from the field sclerotia are nearly mature.

May 15. An apothecium from a sclerotium developed in tube B 21 C (a) 4 is mature, as shown by the appearance of a cloud of spores when the cover was lifted. This particular group of sclerotia was isolated from the others and covered separately.

May 16. The cover was lifted and ejected spores were caught on a poured plate of corn-meal agar.

May 17. Another "volley" was caught, the first being too thickly sown. Single spore isolations were made, these therefore being second-generation ascospore cultures. That is, starting with an ascospore, the culture has gone through its entire life history, gray mold, sclerotium, apothecium, and a new generation started with an ascospore.

Experiment III demonstrates that under favorable conditions of moisture and temperature apothecia can be produced extensively. It also shows that single ascospore strains of the fungus are in themselves capable of producing all the stages of the life history of the fungus—that is, the fungus is purely homothallic.

EXPERIMENT IV.—March 18. In an attempt to influence apothecium production in pure culture, strains 3, 5, 7, 9, 12, 13, and 14 were transferred to new tubes, and the originals, all containing an abundance of sclerotia were submitted to the following conditions:

No. 3 was placed at once in a moist chamber at room temperature.
No. 5 in ice box for 48 hours at 10° to 13° C., then as in No. 3.
No. 7 above a freezing mixture starting at −10° C., overnight, in a well insulated fireless cooker, then as in No. 3.
No. 9 above a freezing mixture overnight, then as in No. 3.
No. 12 in ice box for 14 hours, than as in No. 3.
No. 12 2d in freezing mixture overnight, then as in No. 3.
No. 13 in freezing mixture, with gradual rise to +10° C., 36 hours in all, then as in No. 3.
No. 14 in freezing mixture overnight, then as in No. 3.

The moist chamber was on a shelf in the laboratory, some distance from any window. The cultures were watched for several weeks. No development of apothecia occurred on any of them under those conditions. Finally they were removed from the moist chamber and placed in a culture-tube rack on the laboratory shelf.

Eventually, later in the summer, several sclerotia from these tubes gave rise to apothecial stalks. Included in those so doing were tubes Nos. 9 and 13, which showed that the actual freezing of the sclerotia was in no way injurious to the life of the fungus. Neither did it stimulate apothecium production in any way.

EXPERIMENT V.—April 12. A large number of field sclerotia were placed in Petri dishes of unsterilized moist sand. These were subjected to varying conditions, particularly with regard to temperature. Plate No. 6 placed in a moist chamber at room temperature was the first (May 10) to start to develop apothecia. No. 4 was held until May 24 in a moist chamber in a warm dark room without development of apothecia and on this date was moved out of doors, still maintaining moist chamber conditions. Almost immediately thereafter apothecia began to develop.

Nos. 2 and 5, held in the ice box until May 26, were on this date placed out of doors with No. 4; and also shortly afterward they developed apothecia. On May 26 plates 3 and 6, the former having been held for the first week in the ice box, each had a dozen or so beautiful apothecia.

From this experiment it would appear that high humidity and a warm temperature are the primary requisites for apothecium production. There are strong indications that light as well is an essential factor.

EXPERIMENT VI.—April 15. Several large, black sclerotia from various pure culture tubes were picked out under sterile conditions and placed half buried in sterile sand in Petri dishes. These were subjected to varying temperature conditions as in experiment V. Here, too, the cooling in the ice box and alternate cooling and warming had no beneficial effect, for apothecia were first produced on the plate placed promptly under a bell jar under room temperature conditions. On May 10 sclerotia from strain B 11 showed two large apothecial stalks developing. On May 26, in addition to mature apothecia from B 11 they were found also from strains B 1, B 19, B 21, and B 21C.

In this case mature apothecia were produced from definite single conidium strains of the organism, namely B 11, and B 19, under sterile conditions.

EXPERIMENT VII.—April 17. A large number of sclerotia from castor-bean inflorescences and stalks in the field were placed in sterile sand in Petri dishes and subjected to varying temperature. As in experiments V and VI there was no particular difference in apothecium production in the different lots. On May 10, plate 1, placed at once under warm, moist conditions, showed many stalks arising. Plate 2, placed first in the ice box for a week and then placed with No. 1, also showed several. Plate 3, in a covered Petri dish, showed two or three on May 17. Evidently the lack of aeration had inhibited their development to some extent. On May 26, Nos. 1 and 2 showed dozens of mature apothecia, which were used to swell the collection. No. 3 as well showed several mature apothecia.

At the same time that these sclerotia were started, others were surface sterilized by momentary immersion in 95 per cent alcohol, and then for 3 minutes in 1 to 1,000 mercuric chlorid followed by two changes of sterile water, then placed on a corn-meal

agar plate, and on sterile sand. On April 23 one sclerotium was developing conidia; on May 10 conidia were developing from most of the sclerotia; and on May 26, a sclerotium on the agar was developing an apothecium.

Thus, under sterile conditions, the apothecial stage was produced from field-borne sclerotia.

EXPERIMENT VIII.—April 15. A large number of sclerotia from the field, mostly from Dr. Howell's place at Orlando, Fla., were placed on moist sand in half-liter Erlenmeyer flasks and subjected to a variety of conditions.

May 8. Flask No. 1, placed at once in a moist chamber in the laboratory, showed apothecial stalks arising from two or three sclerotia. Flask 2, which had been placed out of doors under the east side of the building, where it was subject to a little sunlight early in the morning, and during the day to ordinary daylight conditions, with a temperature fluctuating from rather cool at night (approximately 10° C.) to rather warm in the day (approximately 27°) showed several small apothecial stalks arising. Flask 4, after having been held in the ice box for a week, then subjected to room conditions, showed dozens of stalks, none of them as yet mature.

May 12. Flask No. 1 showed many well-formed apothecia. No. 2 showed more than a dozen short stalks with well-formed cups nearly mature. No. 4 showed many well-formed cups. On May 22 these were photographed within the flask (Pl. 11, B), and then removed still attached to the sclerotia and photographed in a Petri dish. (10, Pl. XL). Some of these were then dried in the sun, and others were preserved in alcohol.

May. 24. Flask No. 3, which had been held thus far in the dark under conditions as favorable as any of the others as regards moisture, and in a constant warm temperature, without a sign of the development of apothecia, was placed out of doors under the east side of the house. Almost immediately apothecial development began to occur. On June 2, eight days later, several apothecia as well formed as in No. 2 had arisen.

This experiment, together with the repeated observation that had been made that apothecial stalks were distinctly phototropic, indicated that light is one of the essential factors for the production of apothecia from sclerotia. It also indicated that the use of moist sand in Erlenmeyer flasks was a convenient and favorable method for keeping moisture conditions right. Thereafter this method was used extensively for the production of apothecia.

EXPERIMENT IX.—May 28, 1919. A large number of field sclerotia were collected and planted upon moist sand on a glass plate placed upon the laboratory table, the whole being covered with a bell jar. On June 3, six days later, apothecial stalks are already arising.

The development being so much quicker in this case than ever before indicates a marked seasonal influence. Whether this consists of simply more favorable temperature or of a difference in light duration or of some other factor is not known.

A still later placing of sclerotia, these under absolutely pure culture conditions, the sclerotia having been derived from pure cultures and having been placed on sterile sand, was examined on September 17. Apothecia were produced in abundance from sclerotia of strains B1, B 21, B 35, and B 37, the two latter having been authentic single conidium strains.

EXPERIMENT X.—About the middle of March field sclerotia were collected and subjected to outdoor conditions in a variety of locations in an attempt to produce apothecia under more nearly natural conditions. The first lot of sclerotia was placed on sand in a shallow wooden box, which was subjected to moist conditions by placing on a thick bed of "Spanish moss" (*Tillandsia* sp.) kept saturated with water, and covering the whole with a large moist chamber built of window sash. On April 21 it was noted that this was becoming very hot during the middle of the day, and since apothecia had not yet been produced, the box of sand was removed and placed in the shade of some boxes on the west side of the house and kept moist by waterings from time to time. On April 24 under these more favorable conditions, temperature apparently being a factor, apothecial stalks began to form, and on May 3 mature apothecia were collected

Lot 2 was placed on sand in a small shallow box which was sunken till the top was ush with the ground under the east edge of the laboratory. It was watered occa-onally, but at times the sand was quite dry. Finally, on May 10, a few very small pothecia were found.

Lot 3. On April 15 field sclerotia were placed in marked locations in a castor bean eld under natural conditions. On June 2, after several days of continuously wet eather, a few apothecia were found to have developed.

It would seem that a moist substratum alone is not sufficient but that lso a moist atmosphere is necessary for the best production of apothecia. t would also seem that too high temperature is capable of inhibiting the evelopment of apothecia.

EXPERIMENT XI.—It has been noted in experiment III that single ascospore cul-ures from second-generation apothecia had been made. The development of scle-otia was so prompt in these cultures that it seemed possible to place these sclerotia nder favorable conditions and get still another and perhaps several more generations. arly in July, 1919, this was done. Before the end of the month a third generation f apothecia was produced. A mature apothecium was laid on its side in a poured late of clear agar and left several hours. When the cover was lifted the apothecium hot forth a cloud of spores, which were, of course, caught on the surface of the agar. ingle ascospores were isolated and further cultures started. These, too, produced clerotia which produced another crop of apothecia, this time within 30 days of the ime the single ascospores were isolated. Thereafter this process was continued, at irst in Florida and later in Washington, D. C., whenever time permitted, in some ases after delays of several months. In July, 1920, the eighth consecutive generation f apothecia was produced, but there was then no opportunity to secure single asco-pore isolations. This was accomplished in August. At the same time, following a uggestion from Prof. W. B. Brierley, of the Institute of Phytopathological Research, larpenden, England, who was then a visitor in America, single conidia taken from he seventh-generation single ascospore cultures were isolated to determine whether hey, too, could develop the perfect stage. These cultures were placed in racks in he laboratory. After three months' absence, the writer returned and found that nany of the tube cultures, both single ascospore and single conidium, had produced pothecia in the tubes. These, however, had shriveled and become covered with onidia and mycelium. Consequently in November new single conidium isolations ere made. In addition, castor bean plants in the greenhouse were inoculated with ne seventh-generation strain, the typical disease was produced, and single conidium olations were made, directly from the host. At this time, also, mature eighth-eneration apothecia were available, and single ascospore isolations were made from nem. Transfers of all three strains were made to several tubes of oatmeal agar, and n due time an abundance of sclerotia developed. On December 15, several sclerotia ere transferred from each lot to Erlenmeyer flasks of moist sand. In addition, clerotia that had been collected in the field at Orlando, Fla., in July, 1919, were placed nder like conditions. All the flasks were placed in the greenhouse. After several eeks an examination disclosed that every one of the four strains had borne mature pothecia.

Thus ninth-generation apothecia were procured, each generation after ne first having started from a single ascospore; single conidium strains om two sources both produced normal apothecia; and field sclerotia a ear and a half old did likewise. All four strains were used to start new ngle ascospore cultures according to the usual method. Plate 10, C nd D, were taken from two of these plates.

Eventually tenth-generation apothecia were developed, and ascospores ere sown and isolated from them. In the entire series there appeared o be not the slightest change in appearance in culture, as compared with nore recent isolations, or in ability to develop all the life-history stages.

INFLUENCE OF LIGHT ON APOTHECIUM DEVELOPMENT

In all this work the conclusion could not be escaped that the produc-ion of apothecia was much more readily accomplished in the summer nonths than in the winter. It was very rarely that apothecia were

produced in the culture tubes themselves in the winter time, whereas in both the summers of 1919 and 1920 this phenomenon occurred regularly. That temperature was one factor involved in this seems evident, for t observation was made repeatedly that at the lower temperatures, ev with good light and moisture, apothecium production was slow and t stalks were abnormally thin and the cups not well opened out. It w evident as well that light was involved. Preliminary experiments we started in November, 1920, to investigate this point further.

EXPERIMENT XII.—November, 1920. Mature sclerotia were taken from seve different tubes and evenly divided between two 250-cc. Erlenmeyer flasks, ea containing 100 gm. of sterile sand and 25 cc. water. Thus the conditions were near alike as possible. One of the flasks was then wrapped completely in black pap Both were placed under a bell jar on the laboratory table with a dish of water bene in order to keep the humidity high. A 60-watt Mazda light was placed about inches to one side and was kept lighted every night for several weeks. The nearn of the light was adjusted until the temperature within the bell jar was about 25° which had been shown to be the optimum for development of the fungus. Fo long time no development occurred. After two months, however, numerous a thecial stalks commenced to rise from the sclerotia in the flask exposed to the lig These attained considerable length, in some cases at least 2 cm., and all leaned v strongly toward the source of light. None of them opened out into normal apothe however. In the dark flask no development whatever occurred.

EXPERIMENT XIII.—December, 1920. After this very clear demonstration of influence of light was observed, a further, more extensive series was started, w the cooperation of Mr. H. A. Allard, who very kindly consented to the use of his c trolled light conditions (9) in the greenhouse at Arlington Farm. This was primar a length-of-day experiment, and involved the turning on of electric lights at dusk the evening, leaving them on long enough to make the day the length desired. Lig of different intensities were used, the intensity being determined by the nearn of the light to the subjects of the experiment. Two flasks each, prepared as describ for experiment XII, were placed under four different conditions: (1) a 20-hour d the source of added light being small Mazda lamps near the roof of the greenho several feet away from the flasks; (2) a 20-hour day, the added light being a 100-w lamp about a foot distant from the flasks; (3) a 20-hour day, with a 100-watt lan about 3 feet distant; (4) the normal day for that time of year, approximately 9 hou In addition, a flask prepared like the others in all respects was covered complet with black paper to shut out all light. Temperature and moisture conditions we as nearly alike as could be maintained under ordinary greenhouse managemen The sclerotia in the different lots were from identical sources, and included som from nature as well as from artificial cultures. Observations were made once a wee and for three weeks no change was to be seen in any of the flasks. On the fourt week one flask of lot 3 showed early stages of apothecial formation. Six weeks aft the experiment was started mature apothecia were found in all the flasks subje to increased day length, the largest and best specimens being found in lots 2 and while in the flask under ordinary daylight conditions a few spindling stalks had arise which never opened out. The sclerotia in the dark container had not develop the slightest suggestion of an apothecial stalk.

This experiment again shows the influence of light on the germination of sclerotia, but results are still far from satisfactory. The normal summer development of apothecia was not even approached under the artificial conditions that prevailed. It is to be hoped that some more work can be done along this line.

PERFECT STAGE IN NATURE

The first appearance of the perfect stage under altogether natur conditions was not observed until early in July, 1919. After a prolong rainy period at Orlando, Fla., a careful examination was made of casto bean plants and of the ground beneath in the Boardman field. Sever apothecia were found arising from sclerotia that had fallen to the groun

n addition, several castor-bean stalks were found (July 21 and Aug.) with sclerotia still attached producing apothecia in great abundance Pl. 6, D). It has been seen that each apothecium is capable of discharging spores in a distinctly visible cloud. It is clear, therefore, that, even were the conidial stage not capable of living over the winter, the apothecial stage thus produced would be capable of starting an abundance of primary infections.

CONCLUSIONS FROM EXPERIMENTS

These experiments and observations bring out the following points of interest in connection with the development of apothecia of this organism under controlled conditions:

(1) Moisture. Reasonably moist conditions must prevail, both in the substratum upon which the sclerotia are resting and in the air above.

(2) Temperature. While a wide range of temperature will permit of their development, most rapid growth occurs under fairly warm conditions, averaging about 25° C., the optimum for the vegetative growth of the organism.

(3) Aeration. Provision must be made for access of air to the sclerotia; too small an air space above them, as for instance that which would be found in a covered Petri dish is not as favorable as a greater amount, other things being equal.

(4) Light appears to be necessary, both as an initial stimulus and for continued normal development; those developed with insufficient light are long, slender, and irregular and do not open up normally.

(5) Subjecting sclerotia to freezing, drying, or an extended dormant period previous to placing them under favorable conditions does not favor apothecial development.

(6) The fungus is purely homothallic. Sclerotia from single spore cultures develop apothecia as readily as do those from mixed strains.

MORPHOLOGY AND PHYSIOLOGY OF THE PERFECT STAGE

It is hard to distinguish the apothecium macroscopically from that of any other Sclerotinia. It is smaller, on the whole, than those of the well-known forms *Sclerotinia libertiana* and *S. cinerea*, the largest seen having a disk only 7 mm. in diameter; the stalk normally is only 1 or 2 mm. long. The disk or cup is not so often funnel-shaped as it is with the other two forms, but it is more frequently opened completely or even recurved, as shown in Plate 11, C, and in Plate XI, of the writer's earlier paper (*10*). The color is that of most Sclerotinias and varies somewhat from cinnamon brown to chestnut brown, the stalk sometimes being lighter, sometimes darker. From one to several may be borne upon a single sclerotium.

Any unfavorable condition that may arise while apothecia are forming may cause strange abnormalities in their structure. They often fail entirely to open out, the tip being tubular for a short distance back. Such forms have no normal asci, but rather hyphal tips resembling the ordinary paraphyses. Another abnormality that has been observed after a temporary check in the growth of an apothecium has been a division near the tip into several distinct cups. Sometimes these have failed to mature, and again they have developed several normal mature ascus-producing disks. Plate 11, D, shows an apothecial stalk with five distinct disks.

The discharge of spores from an apothecium was repeatedly observed. This appears to be brought about by a sudden change in the humidity surrounding them. When a good "crop" of apothecia have reached maturity in one of the flasks, as shown in Plate 11, B, or in any other moist chamber in which they have developed, opening the chamber to the air by removing the stopper or otherwise will result in the sudden discharge of a cloud of spores. This phenomenon may not be observed again until the chamber has been closed for several hours and again opened, when it will be repeated.

Many ascospore isolations were made by placing a mature apothecium on its side on a poured plate of clear agar with the disk facing the center. When the apothecium is left there for a few hours, the removal of the cover causes the sudden discharge of ascospores, which may be carried the full width of the plate. After such an occurrence the deposit of spores can sometimes be clearly seen with the naked eye on the surface of the agar, so thick are they. Microscopic examination of one such plate disclosed the interesting fact that often all eight spores from a single ascus when discharged clung together as if in a gelatinous matrix. Large areas on the plate were covered with groups of eight spores. Plate 10, D, is a photomicrograph taken with a Zeiss 2.5-mm. water-immersion objective placed directly over one of these areas on the plate. Ascospore discharge also takes place either intermittently or the spores may be scattered when ejected, for spores may be found on the plate either singly or in groups of two or three. By examining the plate through the bottom with a low-power objective near the edge of the wedge-shaped area in front of the disks, it is an easy matter to locate as many spores as may be desired and mark them for transfer to another plate or a tube.

Ascospore germination takes place very quickly on a plate of agar. Normally it is by the production of a germ tube which continues to grow vegetatively until conidiophores arise, and the gray mold stage is formed, which is usually within 36 hours. In one case, when such a plate as the one just described was held in the ice box over night, it was observed that microconidia were being produced on the ends and sides of comparatively short germ tubes. This condition is pictured in Plate 10, C. The microconidia continued to develop during the day, a succession of them being borne on the end of a single conidiophore, until quite a group of them were to be seen. In no case could germination of microconidia be seen. The same plate shows normal germination of ascospores by germ tubes. This is also shown in various stages in Plate 12, H and I, which consists of camera-lucida drawings of spores from which the first ascospore cultures were derived.

DESCRIPTION OF THE FUNGUS

For the convenience of the reader the complete description of the fungus, as published in 1919 (*10*), is repeated here.

SCLEROTINIA RICINI

Apothecia one to several from a single sclerotium, 5 to 30 mm. high, usually 6 to 15 mm., infundibuliform to cyathiform and discoid, long stipitate, cinnamon brown to chestnut brown; stalk concolorous, cylindrical, slender, smooth, flexuous, attenuated below, without rhizoids; disc at first closed, expanding to saucer-shaped with margin sometimes recurved, exterior roughened, 1 to 7 mm. in diameter, usually 1.5 to 4 mm.; asci cylindrical to cylindro-clavate, apex slightly thickened, opening by

a pore, 50 to 110 μ by 6 to 10 μ, usually 80 to 100 μ by 8 μ; ascospores 8, ellipsoidal, often sub-fusoid, hyaline, continuous, bi-guttulate, 9 to 12 μ by 4 to 5 μ; paraphyses abundant, filiform, septate, hyaline, 1.5 to 2 μ in diameter. Conidial stage (*Botrytis* sp.) forming widespreading cobwebby or somewhat woolly mass, pale drab-gray to drab, dried specimens dark olive-gray; sterile hyphæ procumbent, hyaline, many-septate, often vacuolate, frequently anastomosing; fertile hyphæ long, slender, smooth, slightly constricted at the base, olivaceous when mature, dichotomously branched, terminal branching compact, apices noninflated, thin-walled, collapsing when the conidia fall; proliferation sometimes occurring; conidia borne on sterigmata, globose, smooth, hyaline, 6 to 12 μ, usually 7 to 10 μ, compactly grouped; microconidia globose, hyaline, 2 to 3.5 μ, borne apically on short, obclavate, single or clustered conidiophores that develop on the sides of hyphæ or on tips of special branches; appressoria rare, microscopic, 20 to 60 μ across base. Sclerotia black, rough, elongate, irregular, 1 to 25 mm. in length, usually 3 to 9 mm., suberumpent to superficial, often anastomosing, developing on axes and peduncles of old castor bean inflorescences and on stalks.

Parasitic on *Ricinus communis*, usually on inflorescences, also on stems and leaves; type locality, Orlando, Fla.; distribution Florida, Mississippi, Louisiana, Texas, and Cuba. Type specimens deposited with the Office of Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

ORIGIN OF THE PATHOGENE

Early in the progress of the disease it was suspected that the pathogene had been introduced with the seed. This was not proved absolutely for some time, but much evidence pointed in that direction. For example, the disease was found in nearly every field where Bombay seed had been planted, provided the climatic conditions were favorable for the development of the fungus. One such field was at Baton Rouge, La. A field planted to American-grown seed only a mile or two away was not attacked. Native castor beans had been grown for years in Florida without any report of it, and in 1918 they were not affected until late in the season. Much evidence was found in connection with seed produced in diseased fields in America. The fungus was capable of living over for a long period on the seed. On October 30, 1918, specimens were collected at Alvin, Tex. The fungus was readily isolated, and some of the material was laid away in a sealed envelope. Two months later the fungus was again isolated from this same material. On October 25, waste from a hulling plant at Plant City, Fla., including some light-weight beans, was collected. Two weeks at least must have elapsed since the beans were harvested. The material was placed in a sealed envelope. Six months later the fungus was isolated from this material. Plate 7, D, shows the development of the fungus on one of the castor beans in a poured plate of nutrient agar. Finally, sclerotia 17 months old developed the apothecial stage. This shows that sclerotia carried with the seed are able to reproduce the disease even after a long period.

In addition, many observations showed the close contact of the fungus with the developing seed pods in diseased spikes. A castor bean reaches full size long before it is matured. It is then soft and very succulent and the caruncle is prominent, as shown in Plate 7, A. In such condition it offers no resistance to the penetration of the fungus. Plate 7, B, shows a seed in its pod with the mold growing out of the caruncle and even the seed coat; C shows several such seed with the gray mold distinctly visible; E shows several seed which had been attacked before maturity, with sclerotia attached to the seed coat and the remains of the pods. These specimens were collected under natural conditions in diseased castor bean fields.

The writer did not have an opportunity to study the original imported seed until well into the winter. Then repeated attempts were made to isolate the organism, without success. H. E. Stevens, of the Florida Experiment Station, however, working on this phase of the problem earlier in the fall, succeeded in isolating the causal organism from samples of the imported seed. (Letter of May 9, 1919.) He planted them on sterilized sand, and on a few seed the gray mold developed. Later, the writer made a final examination of some of the Bombay seed in one of the original bags, and a single small sclerotium was found. This was planted on a plate of moist sand under a bell jar, and eventually an apothecium, the perfect stage of the fungus, developed.

Letters written to pathologists in India were at first not completely satisfactory in leading to the solution of the origin of the fungus, the earlier replies stating that it was not known there. A subsequent letter from J. F. Dastur, dated February 15, 1921, brought word that the disease had been found in Mysore, South India, and was under investigation by one of the plant pathologists. More recently (Aug. 13, 1921) a culture was received from India. This culture contained only conidia, which in form and size appeared to be identical with the American form. Later appearances in culture, however, throw some doubt on its identity with our organism. In oatmeal agar the sclerotia are not so abundant, conidia are fewer, conidiophores not so definitely dichotomous, and the development of white mycelium is greater. No perfect stage has developed for final comparison. In spite of this possible discrepancy, the evidence is very strong that the pathogene came from India to this country along with the seed. The fact that it has been discovered there only recently, if at all, is especially interesting, in that it appears to present one more case of an introduced parasitic fungus being more serious in its new habitat than in its old.

INOCULATION EXPERIMENTS

Field observations made the first year, in the course of surveys in the South, made it clear that for successful inoculations a prime requisite would be moisture. After a few preliminary inoculations it became evident that an abundance of moisture was necessary not only for primary infection to take place but for the disease to develop in the host, even after the fungus had penetrated into the interior. The first inoculations were made on seedlings by applying the fungus along with some of the culture medium to different parts of the plant, without injury, and then covering with a bell jar. This resulted almost invariably in success. Within 24 hours positive infections became evident. Plate 8, B, shows distinct lesions on a young seedling, produced by artificial inoculation in the Cornell University plant pathological greenhouse, in January, 1919. Other inoculations made at the same time consisted in the application of conidia to young growing leaves. These were successful unless the plants became dry during the incubation period of the fungus. Material of plants, particularly leaves, thus infected, was fixed for later histological work to determine the manner of penetration of the fungus into the host. Castor bean seeds were also sterilized in calcium hypochlorite, after the method described by Wilson (*23*), and planted in large 10-inch tubes of synthetic agar, with the result that about a dozen castor bean plants in pure culture were secured. Inoculations were later made on these with resulting rapid infections.

Most of the inoculations were made, however, at Orlando, Fla., on developing inflorescences of large growing plants. Since the nature of the plant demanded special methods, these will be given in detail. The method most used was one obtained from J. R. Winston, who used it extensively in his work on citrus diseases. For an inoculation chamber a sheet of ordinary waxed paper was used. First a wad of cotton was wetted and wound about the base of the inflorescence to supply moisture. Then the inoculum was applied in the particular form to be tested, and the whole was promptly covered with the waxed paper in the manner depicted in Plate 8, A. First, the sheet was folded lengthwise about the stem, and the vertical edges were brought together and folded twice with narrow folds and creased firmly. Then the upper part was folded down and brought around the stem and held tightly in place with one or two ordinary paper clips. In this way a tight moist chamber was secured in a very short time. It was thrown away at the end of the desired incubation period.

For inoculations with ascospores, it was necessary to be absolutely sure that no conidia were present. A few preliminary tests on plants under natural conditions made it evident that it would be difficult with ordinary methods to insure sterility of parts of the plant to be inoculated. A waxy bloom covered practically the whole plant, and particularly the inflorescence and prevented the spread of solutions of mercuric chlorid applied for the purpose of sterilizing the surface. This difficulty was overcome by using this sterilizing agent in solution in 35 or 40 per cent alcohol. Immediate and complete contact was obtained in this way, and it was determined that 20 seconds' immersion would not injure the plant. This treatment was followed immediately by dipping the part treated, usually the inflorescence, into freshly drawn tap water, which removed the alcohol and mercuric chlorid, and left the plant evenly wetted, an additional advantage when it came to applying the inoculum.

In May, 1919, no natural infection had as yet become evident in the experimental plantings near the laboratory at Orlando, Fla., where inoculations were made. Conditions were the better, therefore, for reliable results. A series of 10 inoculations, with an equal number of controls, was made on castor bean spikes in different stages of development after the methods outlined above. The plants were not sterilized. Conidia from young cultures in Petri dishes were used for most of the inoculations. Transfers containing mycelium and some of the culture medium were used in others. Eight of the 10 inoculated flower clusters showed, at the end of 48 hours, definite signs of infection. The two failures had dried out, owing to insufficient sealing of the paper moist chamber. None of the controls were infected. Typical inoculated inflorescences, removed from the plants and placed in flasks of water under bell jars in the laboratory, developed a heavy growth of gray mold very similar to its appearance in nature. Those left exposed, out of doors, failed to progress. The rainy season had not yet started sufficiently to develop and spread the disease.

Several more inoculations were made at this time, with different strains of the organism, including reisolations from previous inoculations and cultures derived from ascospores. Practically all of them were successful, and the controls remained healthy.

On May 20, inoculations were made with ascospores as follows: Several apothecia were crushed in sterile water and the resulting suspension of ascospores, asci, etc., were applied to castor bean inflorescences not pre-

viously sterilized in small drops with a platinum loop and in considerable quantities by pouring it into the spike. Seven inoculations were made. Two only were successful, those in which the inoculum was applied by pouring. Isolations made from the infected spots produced typical Botrytis in pure culture. On May 24 a further ascospore inoculation was made. A potted plant was secured, the large interfering leaves were removed, and a half-grown inflorescence was sprayed with sterile water. An Erlenmeyer flask containing a large number of mature apothecia was now taken, the stopper was removed, and the resulting cloud of discharged ascospores was blown, with the aid of a rubber tube which had been immediately inserted into the flask, upon the wet spike, which undoubtedly caught thousands of them. The inflorescence was then covered in the usual way for 48 hours. On May 26 infection was evident on two or three male buds. One of them was removed and the surface sterilized, then used to inoculate a poured plate of agar, with a resulting development of Botrytis stage of *Sclerotinia ricini*. The inoculated spike was then cut from the plant and placed in a moist chamber. In three or four days a copious development of the typical gray mold occurred. (See Pl. 3, C.)

The writer was absent from the station during the month of June, and when he returned in July all his experimental plantings were scatteringly infected. This naturally made any further inoculations more or less uncertain, for it was impossible to determine in advance whether or not an apparently clean spike had been previously infected. However, early in September a few more ascospore inoculations were made, this time by placing well-developed apothecia directly into the sterilized and washed inflorescences in marked locations, holding them in place with paste and covering with waxed paper. Six cases out of 10 inoculated showed infection after the usual two days, traceable apparently to the apothecia. Two showed infection appearing elsewhere than near the applied apothecia, and 2 out of 6 controls were infected. The results of these inoculations therefore would be more or less unreliable were it not for the fact that subsequent examination showed penetrating hyphæ directly traceable to germinating ascospores.

At this time also a considerable number of spikes were inoculated with conidia, and material was fixed at different periods for histological study.

HISTOLOGICAL STUDIES

It has been mentioned in various parts of this paper that materials were fixed and embedded for later histological work. The first consideration was to determine the manner of host penetration. A large amount of material was secured for this purpose, both from seedling inoculations at Cornell University, Ithaca, N. Y., and from field inoculations at Orlando, Fla. Material was fixed at different periods after spore inoculations in Fleming's weak killing fluid. The different lots of material were washed and run up to 70 per cent alcohol in the field and held there for several months, then embedded in paraffin at Washington, D. C., during the winter of 1919–20. Several different kinds of differential stains were tried, but best results were secured with Pianeze IIIb (*21*) and with Fleming's triple stain.

Early stages of penetration appeared to be practically identical with those pictured by Blackman and Welsford (*1*) for *Botrytis cinerea*. The leaves of plants grown in pure culture in 10-inch culture tubes on synthetic agar were inoculated by applying conidia thickly on the surface. Mate-

rial fixed 24 hours after inoculation showed abundant spore germination and early stages of penetration before there was any indication of injury to the parts inoculated. Actual penetration of the cuticle was accomplished, as in the case of *B. cinerea*, by a fine peglike projection from the point of contact of the developing germ tube. Plate 13, A to H, shows camera-lucida drawings of 5 to 7 μ sections of castor bean leaves, showing hyphae penetrating the cuticle. No attempt was made to demonstrate muscilaginous substance at the point of contact of the germ tube. After penetration of the cuticle was accomplished, the fungus advanced quickly into the host, and breakdown and disorganization of the host tissue was rapid and complete. The cuticle remained a barrier to easy penetration of other hyphae for some time, however. Sections of materials fixed 48 hours after inoculation showed a tangled web of germ tubes, with abundant production of appressoria and distinct indentations below many of them, indicating marked mechanical pressure. Plate 9, A and B, shows such indentations. The disorganization of the host tissue beneath, with consequent loss of turgor, has permitted deeper indentation than occurs with initial penetration. The tangled web of hyphae of germinating spores which is very evident in the figures would seem to be in itself a factor in withstanding the backward pressure exerted by a penetrating tip. These sections were very thick, approximately 25 μ, and were photographed to show the abundance of hyphae and their apparent pressure on the host, in this case a male blossom bud. Plate 9, C, shows hyphae of the fungus within such a male blossom bud and the complete disorganization of host tissue.

Sections of nectaries from a castor-bean inflorescence showed the protective cuticle to be continuous over its entire surface. The cuticle appeared in some cases to be even thicker over the nectary than over the surrounding parts of the host, but there is no evidence for a conclusion that this had any influence on the comparative freedom of nectaries from primary infections.

The fact that the seeds are important carriers of the pathogene has already been mentioned. Many observations were made of the presence of the mold on and within the seed. Plate 7, B and C, shows the mold itself growing on the caruncle of castor bean seed. The mold was often seen growing over immature pods and penetrating them. Such materials were fixed and embedded, and the presence of hyphae of the fungus was demonstrated with differential stains. Since such seed may reach the size and appearance of maturity, it is evident that the fungus may be carried by this means, along with healthy seed.

HOST LIMITATIONS OF THE FUNGUS

One of the first considerations, when it was determined that the gray mold was a new organism in America, was to determine whether or not it might prove to be a danger to other crops. At Cornell University, in January, 1919, a number of plants that were available were inoculated under exactly the same conditions that were given the castor beans inoculated successfully at the same time. The common geranium (*Pelargonium* sp.) was more severely infected than any of the others. The growing point of the plant was entirely killed, and several of the younger leaves were attacked at their margin (Pl. 8, C). The organism continued to spread as long as moisture conditions were kept favorable. Reisolations were made and identified. As soon as the moist inoculation

chamber was removed the disease stopped progressing, as it did with the castor bean plants. Besides the geranium, eggplant (*Solanum melongena* Linn.) seedlings became very slightly infected. Among the plants that were entirely unaffected by the organism were tomato, onion, snapdragon, sunflower, string bean, tobacco, and lettuce.

In Florida, during the season most favorable for infection, a few other plants belonging to the Euphorbiaceæ, the family to which the castor bean belongs, were inoculated under the same conditions as those given castor beans. Plants inoculated were five or six each of Jatropha (*Jatropha* sp.), Cassava (*Manihot ultilissima* Pohl.), and Poinsettia (*Euphorbia pulcherrima* Willd.). Slight but definite and characteristic lesions were produced on all of these, but in no case did the organism spread sufficiently to indicate that it might ever become active as a parasite on any of them. At the same time geranium plants were inoculated, and with similar results. All the lesions dried up after moist chambers were removed, even though castor beans in the field were meanwhile actively attacked.

Throughout the summers of 1918 and 1919, during the course of survey trips through the various infected localities, a close watch was kept on all manner of vegetation in the vicinity of diseased castor bean fields for possible natural infection. Other than castor beans no diseased plants were found for which the castor bean gray mold was responsible. The writer considers it safe to state that in his opinion the gray mold organism, fatal as it is to the castor bean crop, need not be considered a menace to any other crop. It appears to be very narrow in its host limitations.

VARIETAL SUSCEPTIBILITY

In order to make a test for possible varietal resistance, a collection of as many different varieties of castor beans as possible was made during the winter of 1918–19. These consisted principally of varieties grown by the Office of Drug and Poisonous Plant Investigations of the Bureau of Plant Industry, at Florence, S. C. Twenty varieties were obtained. These were supplemented by typical "Palma Christi" and a few selections of what appeared to be distinct varieties from the miscellaneous assortment included in the Bombay seed. They were planted in a field surrounded by an abundance of infective material. Results were not as satisfactory as might have been wished, owing to a number of adverse circumstances, but general conclusions can be drawn. Plants of the more ornamental type, with stalk, foliage, and sometimes pods in different shades of red or reddish green, were the most resistant, the pods especially being coarser and obviously tougher. All the smaller, many-branched plants, which by their yield indicated commercial possibilities, showed high susceptibility to the disease. Many of them at the close of the season had as high as 90 per cent of the inflorescences affected, and practically all over 50 per cent. Cross pollination in a castor-bean field probably occurs very extensively. It would require years of work to develop pure strains and then to select and breed for desirable qualities combined with resistance before permanent results in the way of control could be secured.

CASTOR BEAN HULLS FOR FERTILIZER

Castor bean hulls have a high potash content, consequently there was considerable agitation during the winter following the harvesting of the

1918 crop for their use as fertilizer. The one objection to this procedure, in case castor beans had become a staple crop, would have been the widespread distribution of the gray mold that might have followed. This danger might have been overcome by grinding the hulls and mixing them with acid phosphate and other ferilizers at the fertilizer plants. The close contact of the hulls to the strong acid of the fertilizer would possibly have quickly killed the fungus. No investigations to check this up were undertaken, however.

CONTROL EXPERIMENTS

Various plantings of castor beans were made in the spring of 1919 on different areas of vacant ground at Orlando, Fla. The sites were chosen particularly with reference to proximity to infected fields of the previous season's growth. Lot A consisted of an area of about ½ acre cut out in the middle of a large field which had been badly diseased in 1918. The soil was a fertile "hammock-land" type, bordering one of the numerous small lakes in the vicinity of Orlando. It required no fertilization but was prepared by first pulling up and clearing away the remains of last year's plants, then simply plowing and harrowing. This was done the last week in February. The field was divided into 1/10-acre sections for convenience, and plantings were made of various lots of seed during the first week in March. A frost the night of April 2 killed many of the plants which were well up, so a day or two later replantings were made, these being final. Lot B consisted of a portion of a lot 50 by 150 feet, approximately ¼ mile from Lot A, which was the nearest of last year's plantings. Lot C consisted of another piece of ground at a distance of approximately ½ mile from the first field, covering an area 200 by 150 feet in dimensions. This was upon sandy soil and required considerable fertilizer. Plate 2, A, is a general view of this field upon which the greater part of the control experimental work was conducted.

During the summer of 1918, when the disease appeared at its height, the standard fungicide, Bordeaux mixture, was recommended provisionally as a possible means of control (*16*, *17*). This spray has been recommended more or less extensively for that type of trouble, as for instance by Stevens and Hall (*15*) for Botrytis diseases (Botryose) in general. As a rule, however, the only practicable means for controlling Botrytis diseases of crops appears to be the application of sanitary measures, namely, the destruction of diseased parts and of diseased plant débris and thinning or otherwise improving ventilation and doing away with excessively moist conditions. It seemed advisable to give fungicide application a thorough trial for this disease, however, and this was done in lot C during the summer of 1919.

A few preliminary experiments pointed out as a method of procedure the following: The portion of the field to be tested was divided into four sections, each containing nine 150-foot rows. These four sections were to be treated, respectively, with (1) Bordeaux dust, (2) lime and sulphur dust (containing 10 parts sulphur to 90 parts lime), (3) Bordeaux spray, 4-4-50, and (4) lime-sulphur spray, 1–30. The latter was found to be definitely injurious in that strength, so after the first application 1–40 was applied. The first three rows in each section were treated twice a week, on Wednesday and Saturday; the second three once a week, on Saturday; and the third three left untreated as controls. Before starting the experiment the plants were examined thoroughly and all diseased and ripe spikes cut off and dropped to the ground.

The applications were made according to schedule for a period of six weeks, except for slight variations on account of unfavorable weather. The sprays were applied with a knapsack sprayer; the dusts, with a hand-operated dusting machine of the fan blower type, suspended by straps from the shoulder. The applications were very thoroughly made, from both sides, so it is very certain that the spikes were well covered with the fungicides. On Saturday of each week, before the regular treatment was applied, a count was made of all diseased and disease-free spikes. The count included immature spikes that were bursting through the sheath, but none younger than that. In this way an accurate comparison was made of the effectiveness of the different treatments. At the same time all the diseased spikes were cut and allowed to fall to the ground, thus providing rows of mold-free plants for the following week.

In connection with the control experiment a test was conducted according to the method described by Winston and Fulton (24) to determine the persistence on the plant of the copper preparations used. Briefly, the particular methods used were as follows: At the time the counts were being made each Saturday liberal samples of spikes from the Bordeaux dust and Bordeaux spray-treated plots were collected at random in paper bags. The samples included about an equal number of diseased and disease-free spikes. Out of each lot 200 gm. were weighed out and washed thoroughly in 500 cc. distilled water into which 1 cc. of nitric acid had been poured. Any copper sulphate

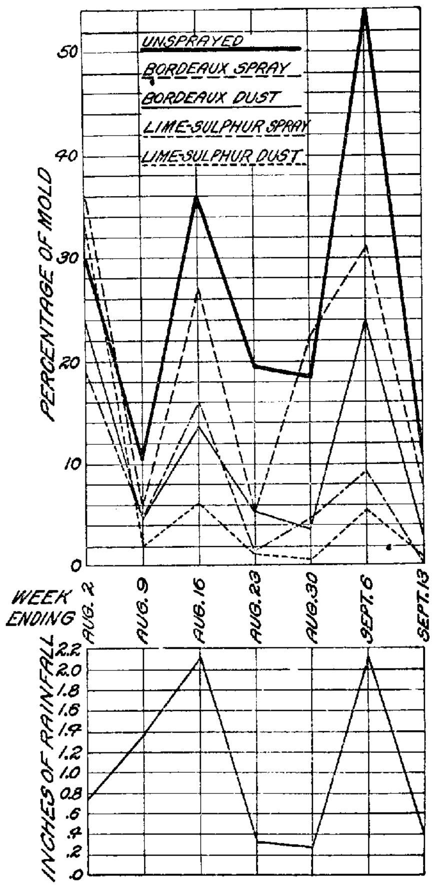

FIG. 4.—Results of spraying experiment from August 2 to September 13, 1919. The graphs show the percentage of mold developing on castor-bean plants, week by week, for each of the fungicides used; and, for the purpose of comparison, the weekly rainfall over the same period.

present was, of course, promptly dissolved. Test-tube samples of these solutions, in duplicate, were then set up in racks and treated with potassium ferrocyanid. The color intensities produced were compared with those in a graded series of copper-sulphate solutions of known concentration, and readings were taken accordingly from the nearest in color in terms which were readily convertible to milligrams of copper per 100 gm. of the sample. It is evident that each unit of weight of such material as castor-bean inflorescences, containing a large proportion of heavy, immature pods, would have much less exposed surface than the same weight in leaves, as given in the paper referred to (*24*); consequently the results obtained in this case are not comparable with theirs. They are comparable, however, within this experiment, one condition with another.

In order to establish a basis of comparison to determine persistence of the different materials on the plants, a special application was made at the close of the series. The spray was allowed to become thoroughly dry and the dusted spikes to blow in the wind for a couple of hours; then samples were collected and tests made for copper, with results as follows: Three of the dusted samples gave 41.7, 62.6, and 50 mgm. copper per 100 gm. of sample, averaging practically 50 mgm. per 100 gm.; two of the sprayed samples gave identical results—25 mgm. copper per 100 gm. of sample. Consequently these figures are taken as very rough bases upon which to calculate percentages of persistence of the copper applications. The results of the spraying experiment, week by week, are given in Table II in figures representing total number, number of moldy, and percentage of moldy spikes. In addition, figures are given showing the weight in milligrams of copper, as such, per 100 gm. of plant parts sprayed, at the close of each week, together with the relation between that and the "perfect" persistence, expressed in percentages. Some of the results are more graphically presented in Figure 4.

Several interesting facts can be ascertained by study of this table and figure 4. In the first place, there is a very definite correlation between rainfall and the development of mold in all the plats. During a week when less than ½ inch of rain fell, as a rule less than 20 per cent of the flower clusters in the untreated plats developed the mold; when over 1 inch fell, more than 25 per cent; and in one week over 50 per cent became moldy. An exception to this was the week ending August 9, when the rainfall was bunched and several days were dry. The difference in the persistence of the copper applications can also be traced, on the whole, to rainfall, though some inconsistencies appear to be present. These are due, as a rule, to the time of application of the fungicides in relation to some of the heavier downpours of rain. For example, during the week ending September 6, the single application of Bordeaux dust almost entirely disappeared, whereas a comparatively large proportion of the second application, made after most of the rains were over (*20*) remained (fig. 5). The copper of the Bordeaux spray was, on the whole, more persistent than that of the dust. The anomaly evident for the week ending September 6, in which the Bordeaux spray has a sticking quality better than 100 per cent, can be explained only by the supposition that an unusually heavy application was made in the middle of the week, after the rain was over for the week.

The comparative efficiency of the fungicides used is best shown in the graph (fig. 4) in which curves are used to represent the percentages of moldy spikes left at the end of each week, under the different treatments. Only the twice-a-week applications are compared here. In so far as control of the mold is concerned, the sulphur applications appear to be

the much more efficient, with the advantage, between the two, in favor of the lime and sulphur dust. However, a burning and consequent serious injury to the plants was very evident with both sulphur compounds. This injury was sufficient to put these preparations out of consideration as practical remedies. The Bordeaux dust, contrary to what might have been expected from the comparisons of relative persistence, was more efficient than the spray. No injury whatever resulted to the plants from the applications of the copper compounds.

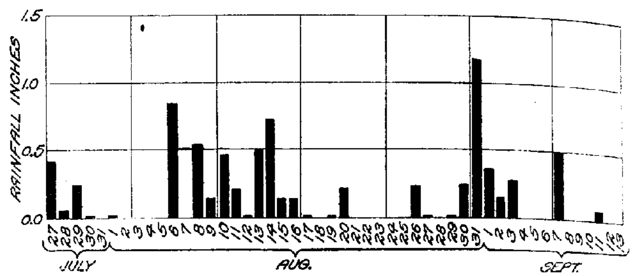

FIG. 5.—Diagram showing the daily precipitation record at Orlando, Fla., over the period covered by the spraying experiment, July 27 to September 13, 1919.

TABLE II.—*Results of applications of fungicides and persistence of copper applications*

Week ending—		Bordeaux dust.			Lime sulphur dust.			Bordeaux spray 4-4-50.			Lime sulphur spray 1-40.		
		Twice.	Once.	Control.	Twice.	Once.	Control.	Twice.	Once.	Control.	Twice.	Once.	Control.
Aug. 2	Total spikes	287	222	208	184	384	272	201	167	110	145	116	46
	Moldy spikes	67	70	61	60	55	68	71	43	37	27	17	14
	Percentage moldy	23.3	31.5	29.3	32.6	19.4	25	35.3	35.7	33.6	18.6	14.7	30.4
	Copper (mgm. per 100 gm. spikes)	5	0	.....	.....	.....	.....	4.2	4.65	.....	.....	.....	.....
	Percentage persistence	10	0	.....	.....	.....	.....	16.8	18.6	.....	.....	.....	.....
9[1]	Total spikes	300	225	206	247	310	262	225	233	171	99	197	82
	Moldy spikes	14	22	16	5	8	19	13	26	19	5	14	13
	Percentage moldy	4.7	9.8	7.8	2	2.6	7.3	5.8	11.2	11.1	5	7.1	15.8
	Copper (mgm. per 100 gm. spikes)	7.8	(2)	.....	(3)	.....	.....	10.45	0	.....	.....	.....	.....
	Percentage persistence	15.4	(2)	.....	.....	.....	.....	41.8	0	.....	.....	.....	.....
16[1]	Total spikes	433	285	311	314	474	450	402	371	235	296	253	112
	Moldy spikes	59	57	129	19	124	134	107	125	83	47	80	40
	Percentage moldy	13.6	20	41.5	6.1	26.2	29.8	26.6	33.7	35.1	15.9	31.6	35.7
	Copper (mgm. per 100 gm. spikes)	6.9	0	.....	(4)	.....	.....	8.3	8.3	.....	.....	.....	.....
	Percentage persistence	13.8	0	.....	.....	.....	.....	33.3	33.3	.....	.....	.....	.....
23[1]	Total spikes	476	335	268	484	324	366	387	339	194	277	124	100
	Moldy spikes	26	50	47	6	23	65	21	48	41	4	10	22
	Percentage moldy	5.5	14.9	17.5	1.2	7.1	17.8	5.4	14.2	21.1	1.4	8.1	22
	Copper (mgm. per 100 gm. spikes)	33.3	4.3	.....	.....	.....	.....	16.7	4.65	.....	(5)	(5)	.....
	Percentage persistence	66.7	8.6	.....	.....	.....	.....	66.7	18.6	.....	.....	.....	.....
30	Total spikes	473	330	278	522	475	366	319	240	193	320	243	91
	Moldy spikes	17	36	34	3	7	51	71	65	36	14	15	26
	Percentage moldy	3.6	10.9	12.2	.6	1.3	13.9	22.3	27.1	18.7	4.4	6.2	28.6
	Copper (mgm. per 100 gm. spikes)	10.4	4.3	.....	.....	.....	.....	17.9	4.3	.....	(4)	(4)	.....
	Percentage persistence	20.8	8.6	.....	.....	.....	.....	71.6	17.2	.....	.....	.....	.....
Sept. 6	Total spikes	528	351	309	399	535	447	488	344	201	319	263	100
	Moldy spikes	126	149	157	22	150	229	151	181	113	30	71	57
	Percentage moldy	23.9	42.5	50.8	5.5	28	51.2	30.9	52.6	56.2	9.4	27	57
	Copper (mgm. per 100 gm. spikes)	33.3	(2)	.....	.....	.....	.....	29.5	4.3	.....	(4)	(4)	.....
	Percentage persistence	66.7	(2)	.....	.....	.....	.....	118	17.2	.....	.....	.....	.....
13	Total spikes	484	335	285	645	571	403	427	309	121	295	222	60
	Moldy spikes	14	24	52	5	38	42	34	32	12	2	4	9
	Percentage moldy	2.9	7.2	18.1	.8	6.7	12	8	11.8	10	.7	1.8	1.5

[1] The spraying was postponed until Monday on account of rain; dusts applied as usual.
[2] Trace. [3] Burning evident. [4] Burned. [5] Much burning.

All the applications used controlled the disease to some extent, as can be seen at a glance. The failure to get anything like complete control was undoubtedly due not so much to the ineffectiveness of the fungicides against the parasitic mold as to the extreme rapidity with which the plants grew and exposed unprotected surfaces to infection by the myriads of spores that were present at all times. New buds would unfold overnight. Young inflorescences would double in size in 48 hours. Male blossoms would open up, uncovering the anthers, thus multiplying the exposed surface many fold and in addition furnishing ideal conditions for infection. Young pods themselves would rapidly increase in size and expose unprotected surfaces. It is evident, therefore, that spraying is out of the question in so far as practical control of the disease is concerned. Three or four sprayings during the season would be impracticable in so far as expense is concerned, to say nothing of the two or more applications per week that would be necessary to produce results.

SEED-TREATMENT

It has been shown that this destructive disease was carried into America by means of the seed and that the seed is perhaps its most important carrier from one section to another. A seed treatment that would destroy the fungus without injuring the seed would therefore be a step toward prevention of the disease. In a seed-treatment experiment carried out in March, 1918, at Orlando, Fla., lots of 200 seeds each were given the following treatments: 1 to 240 formaldehyde (commercial 40 per cent formalin) for 5, 10, 15, 20, 30, 45, and 60 minutes, and murcuric chlorid 1 to 1,000 for equal periods. After the treatments, the seed were planted in rows, under natural conditions. A month later a careful count was made of the germination and growth in each row. The results are given in Table III.

TABLE III.—*Germination of seeds treated with formaldehyde and mercuric chlorid*

Period of treatment (minutes).	Percentage germination.	
	Formaldehyde.	Mercuric chlorid.
5	68.5	73
10	53.5	57.5
15	63	55
20	55	67
30	60.5	63.5
45	64	74
60	67.5	71

A frost on April 1 undoubtedly killed some of the plants, but the results are roughly comparable. For either treatment, germination was practically as high for 60 minutes' immersion as for 5. It is therefore certain that no injury is likely to be done the seed by any reasonable chemical treatment.

Seeds formed in an inflorescence that has been attacked by the gray mold are lighter in weight than normal healthy seed; in fact the worst of them are so light as to be blown out by the blower in a seed-cleaning machine. The castor beans shown in Plate 7, E, are examples, light in weight and worthless as seed. The living fungus inside the tough seed

coat may lie dormant for months. It is very unlikely that any of the heavier seed carry infection inside the seed coat. Simply floating off the lighter seed in water, therefore, would eliminate a large part of the danger of transportation of the disease through this means. Experiments in this connection have shown, however, that a considerable proportion of the apparently normal seed will float in water when first immersed and also that seed which were heavy enough to sink at first may rise later. In a specific trial, half of a lot of 50 seeds floated when first put in the water; in 30 minutes 8 more had risen; after an hour longer 8 were floating and 42 were on the bottom. Several different lots acted the same way, in general. It is recommended, therefore, that, in order to combine the floating process with seed treatment, some such procedure as the following be practiced, if there is chance that the seeds are infected. The seeds should be immersed in water in a large receptacle and left for about 1½ hours with frequent stirring. Then the disinfectant, preferably formaldehyde, should be added in the proper proportion, and the seeds left for 15 minutes longer. At this time all those that are floating should be skimmed off, together with all trash, and discarded in so far as use for seed is concerned. It is true that many good seeds will probably be discarded along with the poor, but they will still be available for oil and the certainty will be the greater that only the best and cleanest seed is reserved for planting.

GENERAL RECOMMENDATIONS FOR CONTROL

Regardless of the freedom of seed from disease, planting castor beans in a locality where the disease is present is almost certain to bring about a diseased crop. Observations made in April, 1921, three years after the war emergency plantings of 1918, showed the disease to be still present and active at Orlando, Fla. Several castor bean plants that had survived the mild winter of 1920–21 and had grown to be trees 15 feet high and 6 inches in diameter at the base were covered with fruiting spikes in different stages of maturity. About 50 per cent of these were affected with the typical gray mold. The first requirement, therefore, toward the growing of a mold-free crop would be planting in a mold-free locality. Planting in locations where climatic conditions are not favorable to the disease would be a still better safeguard, in that no infections could occur from chance introductions of the disease on seed or otherwise. The map (fig. 1) shows the localities in America where the disease occurred and where it did not occur, in 1918. Several States, in some of which in fact, the best yields were produced, are seen to be suitable in this respect. In case the necessity should ever recur for heavy plantings in America, this should be one of the primary considerations. A study of the rainfall maps over a considerable period might be advisable in order to be certain that the 1918 conditions were not exceptional. After choosing the localities where the planting is to be done, the source of seed should be considered. If there is any doubt about the cleanness of seed, 15 minutes' treatment, in either formaldehyde solution or mercuric chlorid, as recommended above, should be given as an additional safeguard. Only as a last resort, to prevent general spread from a limited primary infection, can spraying or dusting be recommended. If accompanied by complete destruction of all the disease that has been found, and if repeatedly and frequently applied, a fungicide might be helpful. But on the whole such measures are not recommended as

practicable. Control measures, therefore, are to be wholly preventive rather than to any extent destructive of the disease after it once gets a start.

SUMMARY

(1) Castor beans were cultivated on a large scale in 1918, as a war emergency measure. A number of insect pests and diseases appeared, the most serious of which was a blight of the inflorescence caused by a new species of Botrytis, the perfect stage of which was later found and described (*Sclerotinia ricini* Godfrey).

(2) The disease was found throughout the State of Florida, and also in Mississippi, Louisiana, and Texas. It was not to be found in fields in South Carolina, Alabama, Georgia, Tennessee, or Arkansas. From 10 per cent up to 100 per cent loss was sustained by growers in the affected districts.

(3) A succession of several continuously wet days was requisite to the development and spread of the pathogene. In localities where there was much of this kind of weather during the growing season the percentage of loss was high; where there was less, the loss was correspondingly lower; where summer rains were of brief duration and followed by long periods of dry weather the disease did not occur.

(4) The disease is a typical Botrytis blight. The inflorescences or "spikes" are attacked, in all stages of development, with heavy growth of mold and complete destruction. The leaves and stems are also attacked occasionally. During the winter and spring following, sclerotia appeared in abundance, at first on the old spikes and later on the stalks of the plants.

(5) The causal organism is very readily isolated, since it is a rapid grower on most of the common culture media and outgrows most contaminating organisms. About 50 isolations were made from different sources, mostly from single conidia. Its appearance in culture is that of a typical Botrytis, with the usual abundance of gray mold and black sclerotia. It is characterized microscopically by its comparatively small globose spores in dense heads and the constant dichotomous divisions of the conidiophores. Appressoria are mostly microscopic. Microconidia occur.

(6) Attempts made to induce a perfect stage to develop during the winter of 1918–19 were at first not successful. Early in March a limited number of cultures showed apothecial stalks arising from sclerotia. Single ascospore strains were isolated from apothecia which matured, and the connection with the Botrytis stage was established. Later a great abundance of apothecia were produced under artificial conditions and were found in nature.

(7) The fungus is homothallic. Freezing, drying, or an extended rest period are not requisite to the development of apothecia from sclerotia. Factors necessary before this will happen are reasonably moist conditions, a temperature near the optimum for vegetative growth of the fungus, a reasonable amount of air space, and light.

(8) The perfect stage is a true Sclerotinia, smaller than the more common forms. Ascospores are discharged forcibly in a visible cloud when the apothecia are subjected to a sudden change from moist to dryer conditions. Frequently all eight spores cling together. Ascospores may germinate directly, with the production of a germ tube and hyphæ indistinguishable from those of the Botrytis stage. Microconidia have been observed on germinating ascospores.

(9) A complete technical description of the fungus is given.

(10) It is proved that the pathogene was brought to America from India with the seed.

(11) Numerous inoculations, reisolations, and reinoculations were made with the use of single conidium and single ascospore strains of the fungus. Most of the inoculations were made by the application of conidia, with the use of special waxed paper moist chambers. In addition infection was secured by the application of ascospores alone.

(12) Histological studies showed that penetration of the cuticle, apparently by mechanical means, was necessary before any injury to the host occurred. After penetration disorganization of the host tissue was rapid and complete. The fungus was found within the caruncle and even beneath the seed coat of seed that had been attacked before maturity.

(13) The fungus appears to be practically limited to the castor bean as a host plant. Of a large list of other plants exposed to infection, only three other plants of the family Euphorbiaceæ and a species of Pelargonium were slightly infected, and infection did not develop further under the conditions that favored rapid spread on Ricinus.

(14) Some difference in varietal susceptibility was noted, the coarse ornamentals in general being more resistant than the commercial seed-producing sorts. Nothing promising in the way of control by this means was evident.

(15) Extensive experiments indicated that control by the application of fungicides was entirely impracticable once the disease was well established.

(16) Castor bean seed are not readily injured by any reasonable chemical treatment. A combination of a floating process, to remove all light-weight seed, with a formaldehyde treatment, would seem to be a practicable means of preventing the introduction of the disease from one locality to another by means of the seed.

(17) Control of this disease in the event of any future heavy planting should consist primarily of prevention. The use of clean seed combined with a judicious choice of districts to be planted, based primarily upon freedon from recent occurrence of the disease, should result in the production of a mold-free crop.

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PLATE 1

A.—Young castor bean inflorescence recently infected with *Sclerotinia ricini*. Note the center of infection with heavy production of gray mold, the very recently infected spots exuding amber-colored drops of liquid, and the variation in stage of development of the flowers, both male and female.

B.—Portion of the stalk of a castor bean plant with sclerotia, developed in the subcortical layers, appearing at the surface.

C.—Axis of an old diseased inflorescence, with the pods long since fallen. Note the sclerotia developing at the base.

D.—Apothecia and sclerotia of *S. ricini*.

Reproduced from painting by J. Marion Shull.

PLATE 2[1]

A.—Portion of an experimental castor bean field at Orlando, Fla., upon which observations were made and experimental sprayings conducted in 1919. Note in addition the cloud effects which are typical of the summer season in most parts of Florida. A shower has just occurred in the distance from clouds shown in the upper left-hand corner of the picture. A storm is rising in the other direction, as evidenced by the large dark cloud shown. Such storms pass over quickly and are followed immediately by sunshine, but their frequency, combined with the relatively high humidity, is conducive to the rapid spread of the gray mold.

B.—A single Bombay type castor bean plant, the majority of whose spikes are affected with the gray mold. De Land, Fla., August 22, 1918.

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[1] All photographs used in illustrating this paper were taken by the author except where otherwise indicated.

PLATE 3

Castor bean inflorescences infected with *Sclerotinia ricini*.

A.—A nearly mature spike, showing typical appearance of the mold under natural conditions. Note drops exuding from new infections on the axis. × 3/4.

B.—A similar spike with heavy production of mold. × 3/4.

C.—A young spike infected by inoculation with ascospores. The mold was developed, after infection occurred, by holding the specimen for 38 hours under a bell jar. Orlando, Fla., May, 1919. Natural size.

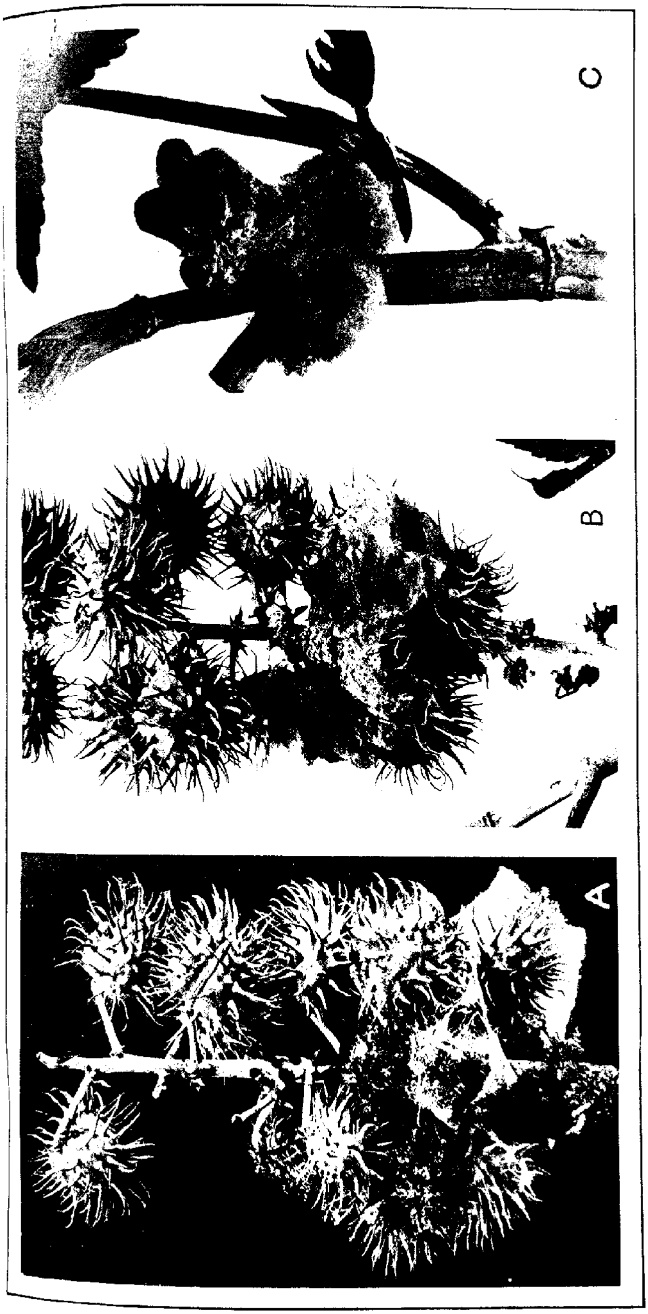
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PLATE 4

A.—Axis of a nearly mature spike almost completely girdled by the fungus. The pods in the center of picture look almost the same as mature pods, but the seeds in them are light and worthless. De Land, Fla., August, 1918. Natural size.

B.—A well-developed spike doomed to destruction, the axis being completely girdled. Note the young spike, also infected. De Land, Fla., August, 1918. × 1/3.

C.—A canker on a stalk, produced by the gray mold. The spike shown was infected when still green and contains worthless light-weight beans. De Land, Fla., August, 1918. × 1/2.

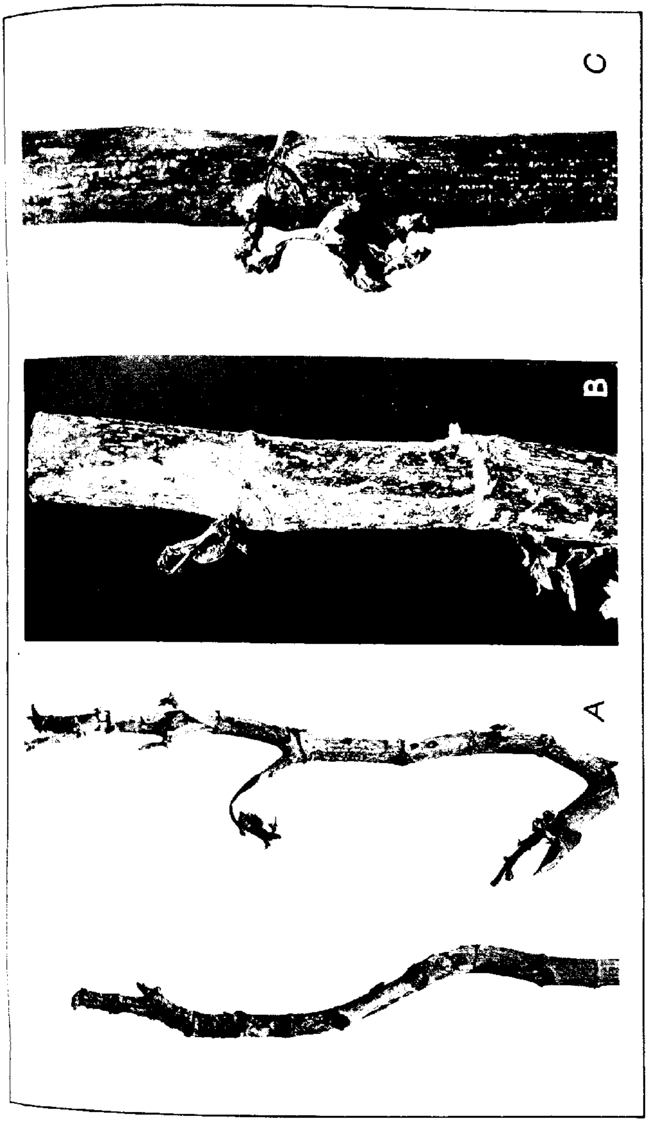
PLATE 5

Winter stage of *Sclerotinia ricini*, Orlando, Fla., February, 1919.

A.—Bases of old diseased inflorescences, showing sclerotia developed in the early spring. × 3/4.

B.—Canker developed on the side of a castor bean stalk from a diseased spur. Note early stage of sclerotium development. × 3/4.

C.—A weak spring growth on the side of an old castor bean stalk. It has become infected with the gray mold and has carried infection into the stalk, producing a canker. × 3/4.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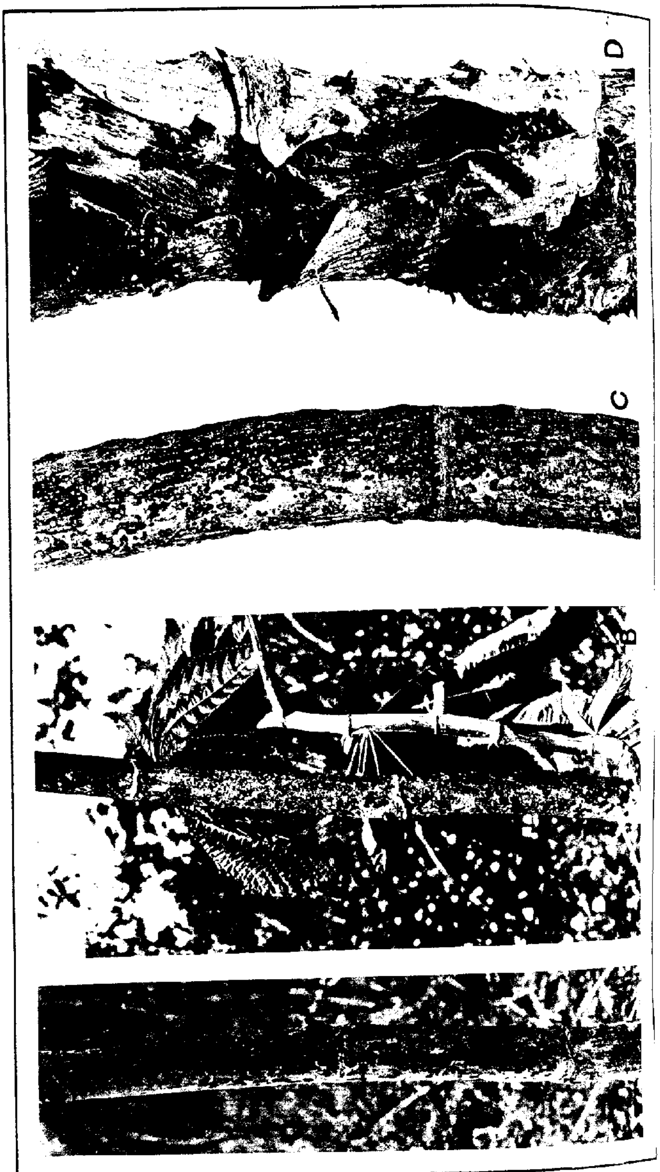
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PLATE 6

Sclerotinia ricini on castor bean stalks, Orlando, Fla.

A.—Sclerotia developed on a stalk, following infection through a spur. May, 1919. × 1/3.

B.—Sclerotia on the surface of a stalk. Note the vigorous, healthy growth developing from below the diseased region. May, 1919. × 1/3.

C.—Superficial and subcortical sclerotia. Note the humps produced by the latter. May, 1919. × 2/3.

D.—An abundance of apothecia developed under natural conditions on sclerotia still attached to the dead stump of the host plant. July, 1919. × 3/4.

PLATE 7

A.—Full-sized but immature castor bean, with part of the hull torn away. Note the large fleshy white caruncle. × 3.

B.—Similar view of a castor bean and hull taken from a diseased inflorescence. Note the gray mold growing on the pod, seed coat, and caruncle. Orlando, Fla. August, 1919. × 3.

C.—Four castor beans from a diseased spike, showing gray mold growing from the seed coat and caruncle. Magnification indicated by the scale.

D.—Gray mold growing from two castor beans six months after they were taken from the hulling plant at Plant City, Fla. The upper one has the mold growing directly on the bean; the lower one shows the mold grown from the seed to the nutrient medium, where it is rapidly developing.

E.—Seed taken from a diseased inflorescence collected at Alvin, Tex., October, 1918. Note the sclerotia on seed coats and hulls. These beans consist of nothing but shells. Natural size.

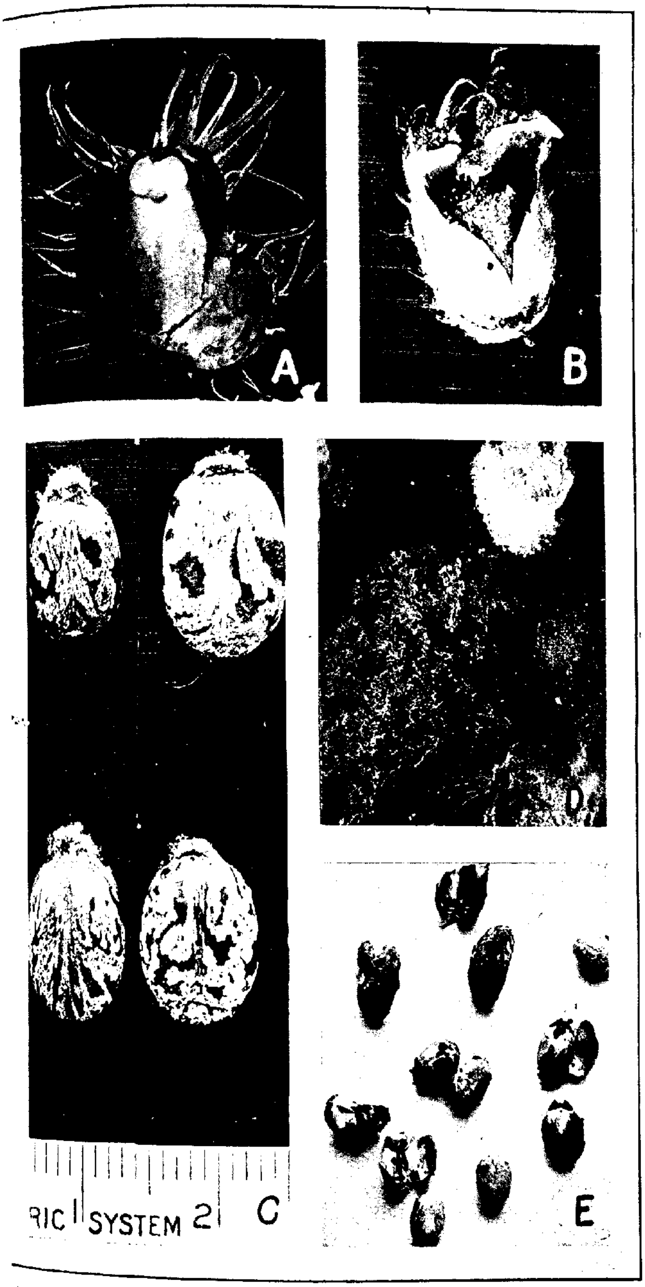
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PLATE 8

A.—Photograph showing the inoculation method largely used in the experimental ork at Orlando, Fla. A sheet of waxed paper is wound about the spike, inclosing moist cotton plug to maintain high humidity.

B.—Portion of an inoculated castor bean seedling, showing lesions on the stem nd attachment of the cotyledons, one of which has already fallen, and the killed rowing point. Photographed by Mr. W. R. Fisher, of the Department of Plant athology, Cornell University. × 7/8.

C.—Geranium infected by artificial inoculation with the castor bean gray mold. te the affected leaf margins, the stem lesion, and the killed growing points. Photo-raph by Mr. W. R. Fisher. × 3/4.

PLATE 9

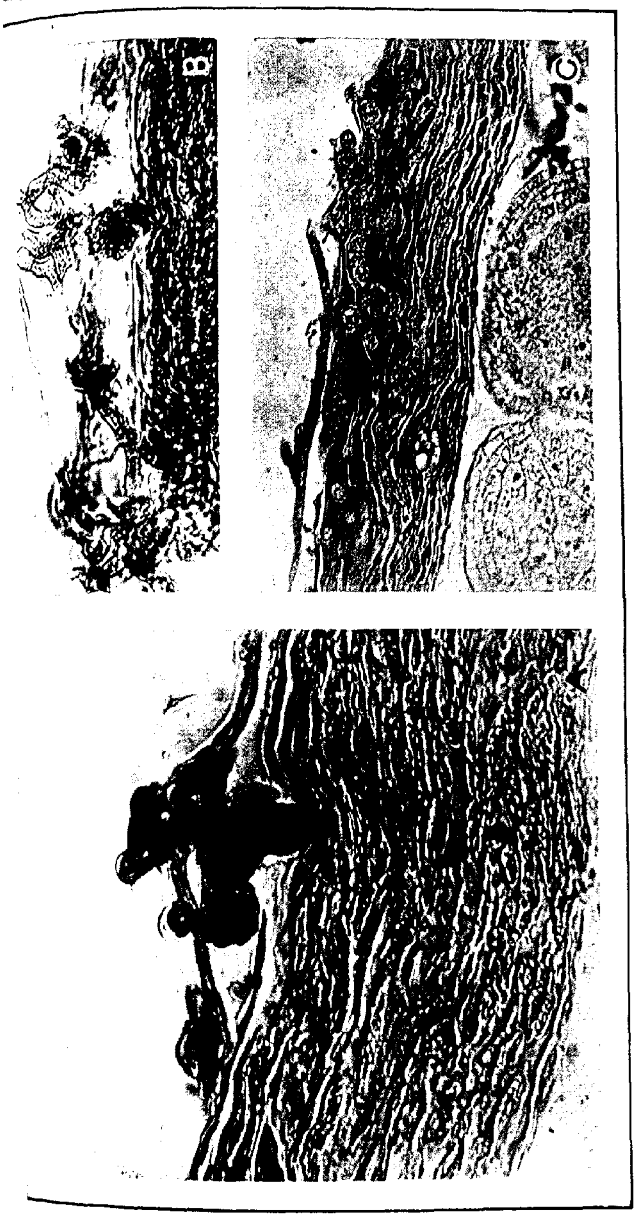
Photomicrographs of late stages of infection of male blossom buds by germinating conidia.

A.—Deep indentation produced by an appressorium. Infection has already occurred elsewhere in the bud and host tissues are disorganized. Section rather thick, about 25 μ. Note clinging together of the hyphae, indicating a tangled web capable of withstanding considerable pressure.

B.—Another view of tangled hyphae, and indentation beneath an appressorium.

C.—Hyphae of the fungus within the disorganized host tissue. The large, round bodies below are partly developed anthers.

Photomicrographed by the photographic laboratory, United States Department of Agriculture.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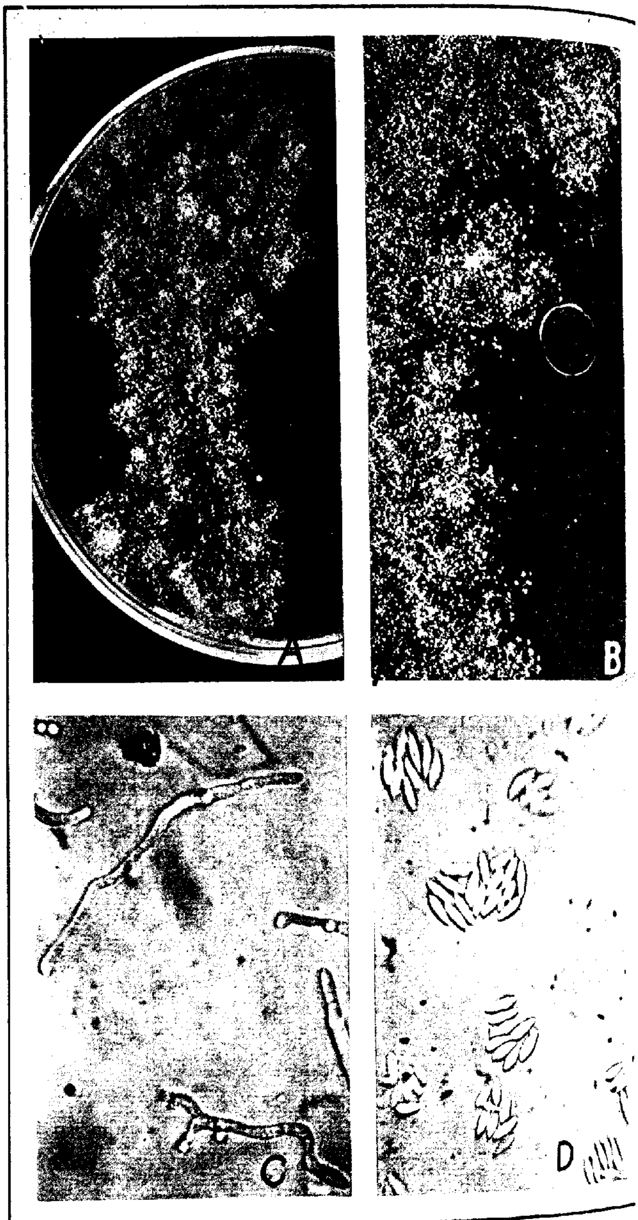
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PLATE 10

Photographs and photomicrographs of *Sclerotinia ricini*.

A.—Gray mold in pure culture on a poured plate of corn meal agar. Culture about 3 days old. Natural size.

B.—Same as A. Approximately × 3.

C.—Germinating ascospores, in some cases with the production of microconidia.

D.—Ascospores as ejected from apothecia in groups of eight.

Photographed by the photographic laboratory, United States Department of Agriculture.

PLATE 11

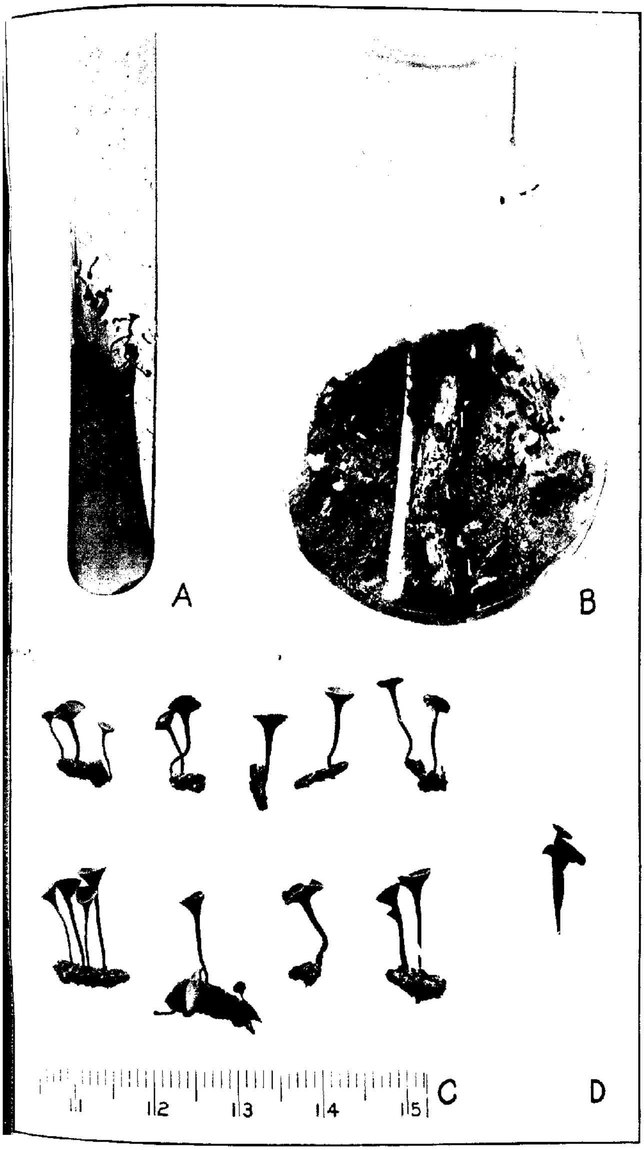
Apothecial stage of *Sclerotinia ricini*.

A.—The first tube in which apothecia developed and reached maturity. The large apothecium in the foreground was the third to mature, and the one from which the first single ascospore isolations were made. Note also the appressoria at *x*. Orlando, Fla., March, 1919. Approximately natural size.

B.—An abundance of apothecia produced from sclerotia placed on moist sand in an Erlenmeyer flask. Orlando, Fla., May, 1919. Approximately natural size.

C.—Apothecia developed as in B, enlarged as indicated, by the metric scale.

D.—An abnormal apothecium with several disks. Somewhat enlarged. Photographed by photographic laboratory, United States Department of Agriculture.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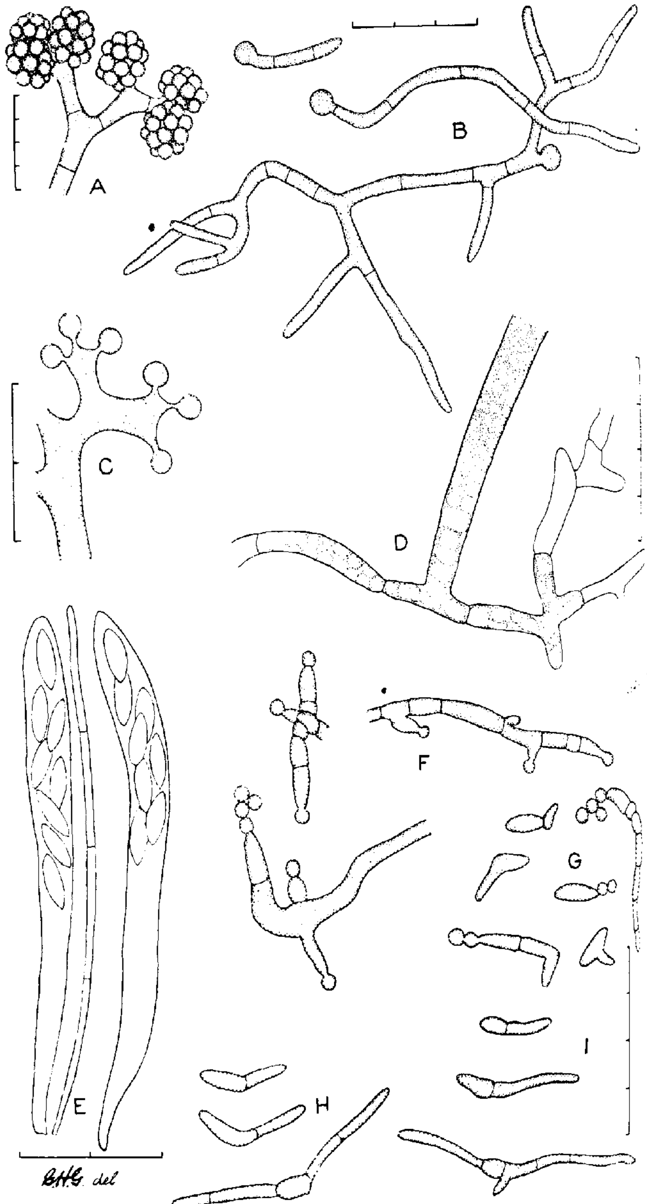
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PLATE 12

Stages in the development of *Sclerotinia ricini*.

A.—Portion of mature head of Botrytis stage. Note dichotomous branching.

B.—Germinating conidia.

C.—Immature conidia, showing attachment to conidiophore.

D.—Base of a conidiophore, hyaline and vacuolate, gradually becoming olivaceous above the substratum.

E.—Asci, ascospores, and paraphysis.

F.—Microconidium production on vegetative hyphae.

G.—The same on germinating ascospores.

H, I.—Stages in germination of two of the ascospores which gave two of the original single ascospore cultures.

Drawings made with the camera lucida. Magnification is indicated by the scales, one space of which, in each case, represents 10 μ.

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PLATE 13

Penetration of a leaf of the castor bean by germinating conidia (except I and J).

A.—Penetration of cuticle by means of a very fine outgrowth almost directly from the spore.

B.—Similar stage of penetration by tip of a germ tube. Swelling of the cuticle seems to be evident.

C.—Two cases of penetration into the cuticle.

D.—A group of germinating conidia. In two places the cuticle has been entered but not completely penetrated. No injury was evident in the epidermal cells.

E.—Another case of partial penetration.

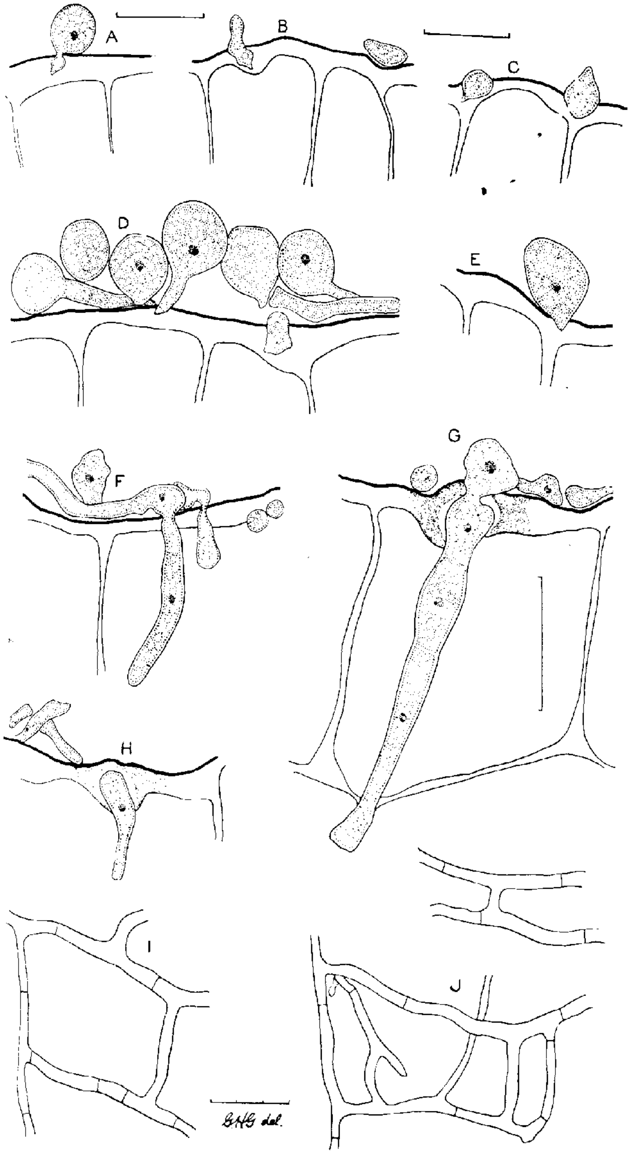
F.—Penetration accomplished and hyphae growing rapidly into the host cells.

G.—Penetration accomplished; the hypha has advanced into the subepidermal layer of host cells. Note the swollen cuticle and the clear region about the penetrating hypha, indicating that some dissolving action has taken place.

H.—Lower part of hypha that has penetrated the cuticle. Apparent dissolving action also seen here.

I, J.—Ansastomosis, or growing together of hyphae in a young culture on clear corn meal agar.

The material was fixed 24 hours after inoculation, some of it showing only very slight injury. Magnification is indicated by the scales, each space of which represents 10 μ.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INFLUENCE OF THE SPECIFIC GRAVITY OF HENS' EGGS ON FERTILITY, HATCHING POWER, AND GROWTH OF CHICKS [1]

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Several interesting theories have been advanced as to the influence which the specific gravity of the hens' egg may have on its fertility, hatching power, and the subsequent growth of chicks hatched. Devices for the purpose of determining specific gravity of eggs are being distributed, claims being made that the hatching power of eggs is directly related to their specific gravity.

Experimental work for the purpose of studying the principles involved rather than for the purpose of investigating any particular device, were conducted at this station with the results as hereinafter stated.

PLAN OF THE EXPERIMENT

The eggs used for this experiment were produced by hens in pedigree breeding pens, use of the trap nest making it possible to watch unusual variations in size, specific gravity, and other characters. The following data were obtained on each of the eggs used in this experiment: Number of the hen laying the egg, weight of the egg to the nearest gram, specific gravity, record of fertility, death in shell if this occurred, and weight of chick if the egg hatched. On the second group of chicks hatched, data were also obtained on the weight of the chicks at 7 days, 21 days, and [illegible] days of age.

The specific gravity of all eggs was obtained by using a device which is sold commercially for this purpose. Actual specific gravity values were determined for each of the divisions on the graduated standard of this device and for the various weights of eggs. Translations were made so that our comparisons and groupings are made on a basis of actual specific gravity.

Each of the 958 eggs used in this experiment was given a distinct number, and all chicks hatched were pedigreed separately. All eggs were hatched in the same kind of incubators, the same operator being responsible for all hatches. The chicks were brooded indoors for the first week and were then moved to a colony house heated with a coal-burning brooder stove.

RELATION OF SPECIFIC GRAVITY TO EVIDENT FERTILITY

The summary of our data as obtained from a study of 958 eggs indicates that there is no correlation between the specific gravity of the egg and evident fertility during incubation. Table I consists of a summary of these data.

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TABLE I.—*Relation of specific gravity to fertility*

Specific gravity.	Number infertile.	Number fertile.	Total
1.01	2	....	2
1.02	1	2	3
1.03	....	3	3
1.04	2	24	26
1.05	12	78	90
1.06	35	178	213
1.07	49	264	313
1.08	40	190	230
1.09	12	56	68
1.10	3	7	1[illegible]
Total	156	802	95[illegible]

$r = -0.038 \pm 0.022$.

Since there is no apparent correlation between specific gravity and fertility, we have eliminated the infertile eggs in organizing data for the next subject of inquiry, which was the relation of specific gravity to hatchability of fertile eggs. Table II summarizes the results of this study.

TABLE II.—*Relation of specific gravity to hatchability of fertile eggs*

Specific gravity.	Number fertile eggs not hatching.	Number fertile eggs hatched.	Total.
1.01	....	....	
1.02	2	....	[illegible]
1.03	2	1	[illegible]
1.04	19	5	[illegible]
1.05	61	17	[illegible]
1.06	112	66	[illegible]
1.07	142	122	[illegible]
1.08	84	106	[illegible]
1.09	24	32	[illegible]
1.10	5	2	[illegible]
Total	451	351	[illegible]

$r = 0.204 \pm 0.023$.

The observations summarized in Table II, made on 802 eggs, with a positive correlation of 0.204, indicates a slight relation between the two factors being studied, but the degree of correlation is so slight that it lacks practical usefulness for the poultry producer.

The relation of the specific gravity of the eggs to the probability of the chick's living through the first five weeks, the critical period, was also studied with the chicks hatched in the second division of the experiment

TABLE III.—*Relation of specific gravity to viability of chicks hatched*

Specific gravity.	Number dying.	Number living.	Total.
1.03	....	1	1
1.04	....	....	....
1.05	....	3	3
1.06	2	15	17
1.07	6	46	52
1.08	8	38	46
1.09	5	23	28
1.10	....	3	3
Total	21	129	150

$r = -0.074 \pm 0.055$.

It has furthermore been stated in commercial literature that chicks hatched from eggs of high specific gravity grow much more rapidly than those hatched from low specific gravity eggs, by virtue of more complete nutritional opportunities in the early life of the high specific gravity chicks. The growth history of 129 chicks were studied with this subject for inquiry, the results being summarized in Table IV.

TABLE IV.—*Relation of specific gravity of eggs to early growth of chicks hatched*

[Expressed in percentage of increase in weight at 35 days over weight at hatch]

Specific gravity.	101 to 200	201 to 300	301 to 400	401 to 500	501 to 600	601 to 700	701 to 800	Total.
1.03	....	....	1	....	....	....	....	1
1.04	....	....	....	....	....	....	....	....
1.05	....	....	....	....	1	1	1	3
1.06	....	....	....	5	4	5	1	15
1.07	....	2	9	18	11	5	1	46
1.08	....	....	2	17	12	6	1	38
1.09	1	....	3	10	5	4	....	23
1.10	....	....	1	....	2	....	....	3
Total	1	2	16	50	35	21	4	129

$r = -0.113 \pm 0.059$.

This observation made on 129 chicks indicates that there is no correlation between the specific gravity of eggs and the early growth of the chick hatched.

FACTORS INFLUENCING THE SPECIFIC GRAVITY OF EGGS

Willard and Shaw[2] report the analysis of large numbers of hens' eggs, ving the following chemical composition: Water, 65.8 per cent; ether xtract, 10.5 per cent; protein, 12.8 per cent; ash in yolk, 1.5 per cent; sh in shell, 9.9 per cent. The specific gravity of the shells of a number f eggs was determined by one of us (Halbersleben) and was found to verage 2.14. The average specific gravity of the whole eggs in this

[2] WILLARD, J. R., and SHAW, R. H. ANALYSES OF EGGS. Kans. Agr. Exp. Sta. Bul. 159, p. 143-177. 09.

group was 1.07, one-half the specific gravity of the shell. The albume constituting approximately 10 per cent of the total egg volume, has specific gravity of 1.31, which is not enough greater than that of t whole egg to have much influence should the protein content of the e vary as much as one-half of 1 per cent, which is the maximum variati reported from analyses of a large number of eggs as reported by Willa and Shaw.[3] The fat, which constitutes approximately 12 per cent the total volume of the egg, has a specific gravity of 0.881 (at 100° C.) Slight variations in the fat content of the egg would not greatly influenc the specific gravity, and the effect of an increased fat content would be lower specific gravity.

Variations in shell weight and thickness are therefore most likely t influence the specific gravity of the entire egg, since the specific gravity the shell is nearly twice as great as that of the albumen and more tha twice that of the fat in the egg. That the shell material has a mo essential function in the nutrition of the embryo in the egg is know for the chick at hatching time has five times as much calcium in i skeletal structure as is present in the egg contents. Harcourt ar Fulmer [5] report (an average of 37 analysis) 0.03615 gm. of calcium ox in egg contents, while the analysis of 65 chicks on the twentieth day the hatch showed a calcium oxid content of 0.19487 gm. Normal eg shells, however, contain at least 2.5 gm. of calcium oxid, so that even thi shelled eggs contain enough calcium to satisfy all requirements, provide other factors influencing its assimilation are satisfactory.

SUMMARY

Our conclusions based on a close observation of 958 eggs and the sul sequent growth of one lot of 150 chicks hatched are as follows:

(1) There is little correlation between specific gravity, fertility, an hatchability of hens' eggs.

(2) The specific gravity of the egg has no relation to the viabilit of the chick hatched or to its growth rate the first five weeks after hatch ing.

(3) Variations in the thickness of the shell are more likely to influence the specific gravity of eggs than are variations in protein or fat content;

[3] WILLARD, J. R., and SHAW, R. H. OP. CIT.

[4] SPAETH, EDUARD. DER NACHWEIS DES EIGELBES (EIDOTTERS) IN MEHLFABRIKATEN. *In* Forsch.-Be über Lebensmittel [etc.], Jahrg. 3, Heft 2, p. 49-54. German abstract *in* Ztschr. Nahr.-Untersuch. [etc.] Jahrg. 10, no. 9, p. 171-173. English abstract *in* Analyst, v. 21, no. 246, p. 233-234. 1896.

[5] HARCOURT, R., and FULMER, H. L. CHEMICAL WORK IN CONNECTION WITH INCUBATION PROBLEMS. *In* Ontario Dept. Agr. Bul. 163, p. 57-66. 1908.

TIME REQUIRED FOR FOOD TO PASS THROUGH THE INTESTINAL TRACT OF FOWLS[1]

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INTRODUCTION

As a preliminary to our study of the digestive coefficients of poultry feeds we wished to run some tests to determine the length of time required for feed to pass the entire length of the digestive tract. The length of the small intestine in a hen of average size is about 61.7 inches, and that of the large intestine 4.61 inches,[2] making a total of 66.3 inches, which, added to the length of the second portion of the esophagus, the proventriculous, and gizzard makes approximately 71.5 inches for the food to pass.

THE PROBLEM

An accurate knowledge of the length of time required for the food to pass the entire digestive tract has a bearing on the time at which it is safe to save excreta from the bird under digestion trial. The problem is to determine just the length of time required for food to pass through the entire digestive tract so that a safe allowance can be made in planning the taking of data of feed given and excreta saved for analyses during the same time.

We also wished to determine whether the rapidity of digestion is the same in growing fowls, broody hens, hens not in laying, and hens in laying.

EXPERIMENTAL METHODS

Fowls of the American breeds were used in these experiments. Only hens were to be tested, and birds 2 to 3 years of age were selected.

The birds were kept in small wire coops 18 inches square with 1-inch chicken netting floor. The coop stood on a tin pan slightly larger than the floor of the coop. This pan was to catch the excreta as it was voided.

Ground feeds, such as wheat middlings and corn meal, were used in which was incorporated a material that would stain the digestive content and indicate just when the food passed out of the body. The indicators were lampblack, methylene blue, and gentian violet.

THE DATA

TEST No. 1.—White Plymouth Rock hen, leg band No. B36, weight, 5.9 pounds.

8 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.

2 p. m. First evacuation; a trace of lampblack appeared.

4 p. m. Still only a trace of lampblack appeared. Fed 20 gm. wheat middlings without lampblack.

6 p. m. Evacuation showing excreta decidedly black.

8 a. m. Second day. 20 gm. middlings and water given.

8 a. m. Third day. After 48 hours excreta showed slight tinge of lampblack on the outer surface but none on the inner particles. 20 gm. middlings mixed with water given

6 p. m. 20 gm. middlings mixed with water given.

8 a. m. Fourth day. After 72 hours excreta normal; no trace of lampblack. Lampblack appeared in 6 hours and disappeared in 72 hours.

[1] Accepted for publication Jan. 16, 1922.

[2] KAUPP, B. F. THE ANATOMY OF THE DOMESTIC FOWL, p. 148, 150, fig. 31, 36, 37. Philadelphia and London, 1918.

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TEST No. 2.—White Plymouth Rock hen, leg band No. 142, weight 6.8 pounds.

8 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
4 p. m. Fed 20 gm. wheat middlings with water and lampblack.
6 p. m. First excreta passed; lampblack stained excreta.
8 a. m. Second day. 20 gm. middlings given.
6 p. m. Fed 20 gm. middlings. Excreta still black.
8 a. m. Third day. 20 gm. middlings given. Excreta nearly normal; only small amount of lampblack on the outside, inside of mass not containing any.
6 p. m. 20 gm. middlings.
8 a. m. Fourth day. After 72 hours excreta normal.

In this test the first voiding of excreta after the feeding of the lampblack was at 6 p. m., or 10 hours. At this time lampblack in small amounts appeared. All lampblack had disappeared at the end of 72 hours.

TEST No. 3.—White Plymouth Rock hen, leg band No. B10, weight, 6.14 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
1 p. m. First evacuation; excreta black.
2 p. m. Second evacuation; excreta black.
7 a. m. Fourth day; excreta free from black.

Lampblack appeared in 6 hours and disappeared in 72 hours. This test was run with a broody hen.

TEST No. 4.—White Plymouth Rock hen, leg band No. 473, weight, 5.14 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
4 p. m. Fed 20 gm. middlings, no lampblack. No excreta were voided on the first day after feeding with lampblack.
7 a. m. Second day. First evacuation; excreta black.
7.30 a. m. Second evacuation; excreta black. This bird acted as though constipated, since no further excreta were voided till morning of the fourth day, or 72 hours. No lampblack was present in these last excreta.

Owing to the fact that no excreta were voided on the day of feeding of the middlings and lampblack, it is impossible to tell how long it required for the lampblack to pass the entire length of the digestive tract. The fact that the excreta were free from lampblack on the fourth day and after the 72 hours showed this test to be in line with those above. This test was run with a broody hen.

TEST No. 5.—White Plymouth Rock hen, leg band No. B148, weight, 6 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
4 p. m. Fed 20 gm. middlings.
7 p. m. Fed 20 gm. middlings.
9 p. m. First excreta voided; excreta black.
7 a. m. Fourth day. Excreta after 72 hours normal; no lampblack.

This test was run on a broody hen. First excreta voided in 14 hours and showed lampblack. Lampblack traces disappeared after 72 hours.

TEST No. 6.—Single Comb White Leghorn hen, in laying condition, leg band No. 34, weight, 2.8 pounds.

7 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
10.30 a. m. First excreta voided; excreta black.
11.20 a. m. Excreta voided; same color as at 10.30 a. m.
7 a. m. Fourth day. After 72 hours excreta normal.

Excreta showed in 3.5 hours that food had passed the entire length of the intestinal tract.

TEST No. 7.—Chick, leg band No. 32, weight, 2 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
10.50 a. m. First excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.

The indicator suggests the food passing through this 2-pound broiler chick in 4 hours.

TEST No. 8.—Chick, leg band No. 36, weight, 1.8 pounds.

7 a. m. 20 gm. wheat middlings given mixed with water and lampblack.
10.55 a. m. First excreta voided; trace of lampblack.
11.40 a. m. Second excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.

In this test the excreta voided indicated that the food had passed through the digestive tract in approximately 4 hours.

TEST No. 9.—Buff Plymouth Rock hen, in laying condition, leg band No. 26, weight, 5.5 pounds.

7 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.

10 a. m. First excreta voided; excreta black.

7 a. m. Fourth day. Excreta normal.

The indicator showed that food had passed the entire digestive tract in 3 hours. An egg was laid the first day of the experiment.

TEST No. 10.—Buff Plymouth Rock hen, in laying condition, weight, 6 pounds, leg band No. 28.

7 a. m. Fed 20 gm. wheat middlings with water and lampblack.

10.05 a. m. First excreta voided; excreta black.

7 a. m. Fourth day. After 72 hours excreta normal.

The indicator showed that food passed through the intestinal tract in approximately 3 hours. This hen laid one egg on the first day of the experiment.

TEST No. 11.—Buff Plymouth Rock hen, weight, 6 pounds, leg band No. 30, in laying condition.

7 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.

10.00 a. m. First excreta voided; lampblack present.

3.30 p. m. Third day. All trace of lampblack gone.

The hen laid an egg each of the first two days. The indicator showed that food had passed the entire length of the digestive tract in approximately 3 hours. It was noted in the latter part of this experiment that the outside of the fecal mass was black and the inside not colored. This raised the question as to whether lampblack will adhere to the intestinal mucosa and be gradually eliminated by the feces as it passes along. In the small intestines where the food is mixed by the pouring back and fourth process, it evidently would be mixed with the food. With these ideas in mind, it was decided to try some tests with aniline blue dyes, which would stain the feeds but not be in a form to be carried along mechanically as was the lampblack.

TEST No. 12.—Columbian Wyandotte hen, leg band No. 26, weight, 5.4 pounds.

7 a. m. Fed 5 gm. whole corn soaked in gentian violet water.

4 p. m. Given 20 gm. soaked in gentian violet water.

7.30 a. m. Second day. First evacuation. Excreta watery in consistency and greenish in color, indicating at first a tendency to constipation and later diarrhea.

This test was considered unsatisfactory.

TEST No. 13.—Columbian Wyandotte hen, leg band No. 28, weight, 5.6 pounds.

7 a. m. Fed 4 gm. whole corn soaked in gentian violet water. There was no evacuation during the day.

8.45 a. m. Second day. First evacuation watery in consistency and greenish in color.

This test was unsatisfactory for same reason as No. 12.

TEST No. 14.—Columbian Wyandotte hen, leg band No. 31, weight 6 pounds.

7 a. m. Fed 3 gm. whole corn soaked in gentian violet water.

6 p. m. First evacuation, greenish and watery in consistency. The constipating effect was not so great as in the two previous cases.

This test was considered unfavorable and unsatisfactory.

TEST No. 15.—Columbian Wyandotte hen, leg band No. 32, weight 5.7 pounds.

7 a. m. Fed 10 gm. whole corn soaked in gentian violet water.

9 a. m. Second day. First evacuation. Feces greenish and watery. The hen appeared weak. Again the gentian violet appeared to cause constipation followed by diarrhea, apparently due to irritation of the mucosa of the bowel.

This test was considered unsatisfactory.

TEST No. 16.—Buff Plymouth Rock hen, leg band No. 26, weight 5.8 pounds.

8.30 a. m. Fed corn meal mixed with methylene blue water.

11.40 a. m. First excreta voided; excreta blue.

7 a. m. Fourth day. After 72 hours excreta normal.

Food passed through the intestinal tract in 3 hours and 10 minutes. There was some irritation. This bird was in laying condition.

TEST No. 17.—Buff Plymouth Rock hen, leg band No. 28, weight 6 pounds.

8.30 a. m. Fed 35 gm. corn meal mixed with methylene blue water.

11.00 a. m. First excreta voided. Excreta were blue. There was some irritation at this time, evidenced by slight watery condition of the excreta.

8.30 a. m. Fourth day. After 72 hours all traces of the blue had disappeared.

Food passed through the digestive tract in 2 hours and 30 minutes. This bird was in laying condition. There was again evidence of irritation.

TEST No. 18.—Buff Plymouth Rock hen, leg band No. 30, weight 5.9 pounds.

8.30 a. m. Fed 8 gm. corn meal mixed with methylene blue water.

9.00 a. m. First excreta were voided; only very slight traces of methylene blue could be detected.

4.30 p. m. Second evacuation; excreta blue.

The first passage was in 30 minutes and indicated that if food is given on any empty crop and mixed with methylene blue slight staining of the food in the cloaca may occur in that space of time. The reaction was constipation followed by looseness of the bowels, as with the gentian trials. At the end of 72 hours no trace of the methylene blue could be detected.

TEST No. 19.—Buff Plymouth Rock hen, leg band No. 32, weight 5.7 pounds.

8.30 a. m. Fed 30 gm. corn meal mixed with methylene blue water.

11.40 a. m. First excreta were passed; excreta blue

8.30 a. m. Fourth day. After 72 hours excreta normal.

The first indication was at 3 hours and 10 minutes. There was again evidence o irritation. The hen was in laying condition, having laid two eggs during th trial.

TEST No. 20.—Buff Plymouth Rock hen, leg band No. 34, weight 6.1 pounds.

8.30 a. m. Fed 31 gm. corn meal mixed with methylene blue water.

8.30 a. m. Fourth day. After 72 hours excreta normal.

Indicator showed food passed entire digestive tract in 3 hours and 30 minutes. Hen was in laying condition. Methylene blue caused irritation.

TEST No. 21.—Buff Plymouth Rock hen, leg band No. 36, weight 6.3 pounds.

7 a. m. Fed 7 cc. methylene blue water mixed with corn meal.

7.30 a. m. Second day. First evacuation; excreta blue.

7.30 a. m. Third day. After 48 hours all blue had disappeared.

This bird was not in laying condition. The first effect was that of constipation the first evacuation being 23 hours after the test meal was given.

TEST No. 22.—White Plymouth Rock hen, leg band No. 115, weight 6.5 pounds.

7 a. m. Fed 20 gm. corn meal mixed with lampblack.

10.20 a. m. First excreta voided; excreta black.

7 a. m. Fourth day. After 72 hours excreta normal.

This hen was in laying condition, laying an egg on each of the three days of th test. The food, as indicated by the lampblack, passed the entire length the digestive tract in 3 hours and 20 minutes.

TEST No. 23.—White Plymouth Rock hen, leg band No. 65, weight 5.8 pounds.

7 a. m. Fed 20 gm. corn meal in which was incorporated lampblack.

12.20 p. m. First excreta voided; excreta black.

7 a. m. Fourth day. After 72 hours excreta normal.

The first colored excreta were passed in 5 hours and 20 minutes. The hen w in laying condition, laying each day of the test.

TEST No. 24.—White Plymouth Rock hen, leg band No. 100, weight 6.3 pounds.

7 a. m. Fed 20 gm. corn meal mixed with lampblack.

2 p. m. First excreta passed; excreta black.

7 a. m. Fourth day. Excreta normal.

The first excreta were voided in 7 hours. This hen was broody. Broody hen apparently do not have as many evacuations as other hens and the quantit evacuated is greater, as indicated by this hen.

TEST No. 25.—Partridge Plymouth Rock hen, leg band No. 112, weight 5.3 pound

7 a. m. Fed 20 gm. corn meal in which was incorporated lampblack.

1.20 p. m. First excreta voided; excreta black.

7 a. m. Fourth day. After 72 hours excreta normal.

The first excreta indicating lampblack was voided in 6 hours and 20 minute

DISCUSSION

Of the two White Plymouth Rock hens not in laying condition, on passed wheat middlings in 6 hours and the other in 10 hours, or a average of 8 hours.

In the test with one Single Comb White Leghorn, in laying conditio the wheat middlings passed through the intestinal tract in 3½ hour Of the three Buff Plymouth Rock hens, in laying condition, two pass wheat middlings in 3 hours and the third in 3 hours and 5 minutes. the two White Plymouth Rock hens laying during the tests, corn me passed through the digestive tract of one in 3 hours and 20 minut

and of the other in 5 hours and 20 minutes, or an average for these two of 4 hours and 20 minutes. An average of the six tests with laying hens showed that food passed the entire digestive tract in 3 hours and 46 minutes.

Of the three White Plymouth Rock hens that were broody, one passed the wheat middlings through the intestinal tract in 6 hours, the second in 24 hours, and the third in 14 hours, or an average for these three of 14⅔ hours. Of two White Plymouth Rock hens in a broody condition, given corn meal, one passed food through the digestive tract in 7 hours and the other in 6 hours and 20 minutes, or an average for the five broody hens of 11 hours and 45 minutes.

Of the two chicks weighing approximately 2 pounds each, one passed the wheat middlings through the intestinal tract in 3 hours and 50 minutes and the other in 3 hours and 55 minutes, making an average of 3 hours and 52 minutes.

Of the two chicks weighing approximately 2 pounds each, one passed the wheat middlings through the intestinal tract in 3 hours and 50 minutes and the other in 3 hours and 55 minutes, making an average of 3 hours and 52 minutes.

Of four Columbian Wyandotte hens not in laying condition, one consumed 5 gm. whole corn soaked in gentian violet water and voided first excrement in 24 hours; the second consumed 4 gm. and passed the first tinted excreta in 25 hours; the third ate 3 gm. and passed the first tinted excreta in 26 hours. From this it is evident that gentian violet causes constipation followed by a looseness of the bowels indicating irritation to the mucosa of the bowel.

Of six Buff Plymouth Rock hens in laying condition, two consumed 30 gm. whole corn soaked in methylene blue water and voided the first tinted excreta in 3 hours and 10 minutes; the third consumed 35 gm. whole corn and voided tinted excreta in 2 hours and 30 minutes; the fourth consumed 31 gm. corn meal and voided tinted excreta in 3 hours and 30 minutes; the fifth consumed 8 gm. corn meal and voided the first tinted excreta in 1 hour and 30 minutes; the sixth consumed 7 gm. corn meal and voided the first tinted excreta in 23 hours.

In these tests there was a tendency for small amounts of methylene blue to cause constipation and larger doses to cause irritation with specks of blood on the semiliquid evacuations. There was only one exception to this tendency and that was the bird that consumed 8 gm. of corn and evacuated in 1 hour and 30 minutes.

SUMMARY

Digestive processes of the fowl are rapid. The greatest rapidity is shown in the laying and the growing fowl, the passage of food requiring on an average 3 hours and 52 minutes for growing fowls and 3 hours and 46 minutes for laying hens. Next in activity comes the adult hen not in laying condition, requiring 8 hours, and then the broody hen, requiring an average of 11 hours and 44 minutes.

It was noted that broody hens behaved the same in the experimental coops as on the nest; that is, the evacuations were fewer and the quantity evacuated each time increased over that of a normal hen.

We have not taken as accurate the fowls in which gentian violet or methylene blue were given, as it exercised influence on the normal function of the intestinal tract.

In all these tests the hens were placed in the coops the day before the trial so that the crop was empty when the test feed was given.

EFFECTIVENESS OF MULCHES IN PRESERVING SOIL MOISTURE[1]

By F. S. HARRIS,[2] *formerly Director and Agronomist*, and H. H. YAO, *Fellow in Agronomy, Utah Agricultural Experiment Station*

INTRODUCTION

The effective preservation of soil moisture is one of the vital problems which confront the dry farmer and the scientist. Any cultural method that has the power of conserving moisture in the soil is a great asset toward solving the dry-farming problem. But the question of the effectiveness of mulches has been much disputed. While in a majority of cases scientists have reported favorable results with the application of mulches, under different conditions the same mulches have not answered the purpose. Indeed, the literature pertaining to this subject presents such varied opinions that it inaugurates complexity into the significance of mulching. The need of a more thorough study of the problem led to the work reported in this paper. Literature on this subject is much scattered, and no attempt has been taken to include all in this article. Only those statements that are closely related to the subject matter have been briefly summarized. The results of the following experiments are tabulated from averages of thousands of measurements.

HISTORICAL REVIEW

OBJECT OF MULCHING

The object of mulching is to preserve a uniform degree of moisture 36)[3] and to hold more water in the soil, the effectiveness of which is rought about by diminishing the direct influence of the agencies of vaporation and by retarding the capillary rise of water to the surface *22, 48*).

EFFECTIVENESS OF MULCHING

The pioneer on mulch work was Wilhelm (*46, 47*), who observed that oils shaded by growing plants contain the least moisture, others covered rith stones and other lifeless objects contain most, while bare soils tand intermediate. This has been confirmed by later workers such as ing (*20, p. 105*), who stated that mulched soils contained more moisture) the depth of 3 feet than rolled soils, Wollny (*48, p. 854–859*) and Iarris and Turpin (14), who found that the effect of mulches was noticeble several feet below the surface of the ground but that the surface oot showed the greatest benefit. Field investigations by Kedzie (*16*) ndicate that to a depth of 16 inches cultivated plots had 3 per cent nore moisture than naked fallow.

[1] Accepted for publication Oct. 19, 1921.
[2] Dr. F. S. Harris resigned from the directorship on Sept. 1, 1921.
[3] Reference is made by number (italic) to "Literature cited," p. 740–742.

MULCH MATERIALS

Any lifeless object can be used for mulching (*46; 47; 48, p. 854–859*). Warington (*42*) has said that the evaporation from the soil may be considerably diminished by protective coverings, such as stones, earth mulch, farmyard manure, straw, dead leaves, or coconut fiber. Clover hay (*17*), straw (*8*), hay (*10*), seaweed (*33*), shingle edgings (*13, p. 24–26*) sawdust (*13, p. 24–26*) and grass (*17; 34, p. 91–93; 39; 41*) had been used for experimental purposes with favorable results on the yield of crops. It was reported by Shimm (*39*) that barnyard manure was more effective than straw or fresh manure, in that it held more moisture and secured a more uniform moisture content in the soil to the depth of 4 feet. Halsted (*10*) used fresh hay, salt hay, and excelsior for mulching, but he failed to find any appreciable difference in the yield of peppers, egg plants, cucumbers, tomatoes, beets, or beans. Munson (*32*) concluded that shingle edgings were excellent for mulching either apple trees or strawberries, but that a fine dust cover could not be excelled for gooseberries. Harris and Turpin (*14*) found straw mulch to be better than 2-inch cultivation.

DEPTH OF MULCHING

Laboratory experiments on the evaporation of moisture from the soil under the mulches proved that the effectiveness increases with the depth (*9, 24*). Under the field conditions the same does not follow the general rule. Sanborn (*35*) and Cardon (*6*) recommended shallow cultivation in preference to deep cultivation. King (*21*) found the soil below a 3-inch cultivation to be more moist than that below 1.5-inch cultivation, although the third and fourth feet showed reversed results, which are confirmed by Chilcott and Holm (7). Shinn (*39*) reported a 3-inch barnyard manure mulch to be more effective than a 5-inch earth mulch, 6-inch straw mulch, or 3-inch fresh manure mulch. Investigations by King (*24*) showed that shallower depths of stirring are to be preferred to the deeper for long-interval cultivations. That a deep mulch maintained during a season of small rainfalls may waste more water than is saved on account of the fact that small rainfalls are wholly retained in the upper layers of the mulch and lost directly and completely to the air, whereas if the mulch has been thin enough to wet through, capillarity would draw a portion of the water downward into the undistubed soil to render effective service when conserved by subsequent cultivation. The work of Burr (*5, p. 53–84*) showed straw mulches, deep cultivation, and shallow cultivation to rank in effectiveness in the order named.

EVAPORATION OF MOISTURE FROM DIFFERENT SOIL CLASSES

As the soil particles diminish in size there is a large increase in the quantity of moisture brought to the surface (15). This is merely due to the surface distribution of water. Meister (*30*) has pointed out that the amount of water retained by soils depends upon the coarseness or fineness of the particles; the less the internal surface of the mass, the smaller will be the proportion of water retained. When fully saturated with water, sandy soils contain 30.4 per cent of water; clay, 38.5; chalk soil, 39.2; loam, 45.4; and garden earth, 76.8. King (*18, p. 197; 19, p. 152*), Loughridge and Hilgard (*26, p. 80–91*), and Lawes and Gilbert (*25, p. 110*) have obtained somewhat similar results.

MOVEMENT OF SOIL MOISTURE

Soils have a great attractive and adhesive force for water (*2*). The retention of water on the surface of soil particles, despite gravitation, is due to surface tension (*3*). The surface of the film of water encircling a soil particle is in an elastic condition exerting a considerable pressure. If the films of water became thicker and heavier, a part of the water would gradually pass out, and if the films became thinner they would acquire the power of absorbing and retaining fresh supplies of neighboring water. Briggs and Lapham (*4*) have attributed the cause of the movement of soil moisture to viscosity, concentration of solution, surface tension, and moisture film curvature. Lynde and Duprè (*28*) believe that osmosis may cause a considerable movement. King (*23*) and Alway and Clark (*1*) have demonstrated the movement of moisture in the soil. Water always tends to move from the wet to the dry soils (*14*). Briggs (*3*), Briggs and Lapham (*4*), Widtsoe and McLaughlin (*45*), and Loughridge (*27*) have shown that the final distribution leaves the most moisture nearest the source of supply and the least farthest away.

EFFECT OF CULTIVATION ON THE TRANSPIRATION OF PLANTS

Slëskin (*40*) and Schroeder (*37*) have determined the water requirement of plants under cultivation by growing beets in cultivated and cement-covered plots. They found the proportion of beet crop grown under cultivation to that which received the cement-covered treatment to be 26.9 to 16.1. At the end of the experiment, the soil under the cement-covered plot contained more moisture than that under the cultivated plot. Experiments by Widtsoe (*43 p. 14–23*) led to the opposite conclusion. The transpiration ratio was invariably smaller on the cultivated than on the uncultivated soils. On the college loam, the ratios on the cultivated and uncultivated plots were 252 and 603; on the sandy clay, 428 and 535; and on the infertile clay, 582 and 750. The favorable effect of cultivation was shown in the great reduction in the water cost of dry matter resulting from simple tillage.

As a general rule, the more water offered the plant the larger the total yield of dry matter (*38, 44*). Mayer (*29*) found that the yield increases with the increase in soil saturation up to a certain point, after which there is a strong diminution in the yield of dry matter. Harris (*11*) and Morgan (*31*) have proved that the transpiration ratio increases as quantity of water added to the soil increases—that is, the water cost of crops becomes larger as more water is used.

TIME OF TILLAGE

Harris and Jones (*13, p. 24–26*) collected moisture samples from the Nephi substation showing that the moisture content of the fall-plowed and spring-plowed plots receiving like treatments was practically same in all cases. The percentage moisture of the spring-plowed plots increased slightly with depth of cultivation, while the fall-plowed plots slightly decreased inversely with depth of cultivation. Straw mulch on the fall-plowed land is the most effective. In comparing fall plowing with spring plowing, Harris, Bracken, and Jensen (*12, p. 30–37*) did not find any material difference in the yield of Turkey wheat. Cardon (*6*) has shown

that the 4-year average moisture content was higher in the spring-plowed plots; but the first 2 feet of the fall-plowed plots contained a higher percentage of moisture.

MULCH VERSUS NO MULCH

In order to demonstrate the effectiveness of mulching in preserving moisture in the soil, an experiment was carried out by using sand immersed in water. The sand was of uniform physical structure, passed through a 100-mesh sieve. It was placed to the depth of 3 inches in a round can, 6 inches in diameter. The bottom was perforated, so that when the can was immersed in water, the soil could be completely saturated through its capillary system. The experiment consisted of free water surface, bare sand, and a 1-inch straw mulch. Each set was performed in triplicate to eliminate experimental error. The experiment extended from April 5 to May 7, and during these 33 days the loss of moisture is recorded in Table I.

Taking the moisture lost from the bare sand as 1,000 per cent, then that which is lost from the soil under the 1-inch straw mulch amounts to 40 per cent. In fact, between the free water surface and bare sand there is only a difference of 8 per cent in favor of the latter. Thus it is to be concluded from this experiment that a 1-inch straw mulch, otherwise under identical conditions, is capable of preserving 60 per cent more moisture in the soil than without mulching.

TABLE I.—*Loss of moisture during the 33-day test*

Can No.	Free water surface.	Bare sand.	Straw mulch.
	Cc.	*Cc.*	*Cc.*
1	1,425.3	1,160.5	480.4
2	1,323.7	1,144.3	439.7
3	1,404.2	1,102	437.9
Average loss	1,384.4	1,135.6	452.7

EFFECTIVENESS OF DIFFERENT MULCH MATERIALS

With the effectiveness of mulching ascertained, the question of the relative effectiveness of mulch materials naturally arises. Almost any lifeless material can be used for mulching, but the relative effectiveness of the different mulch materials varies according to their physical and chemical properties. For the purpose of comparison, five kinds of mulch materials were used—fresh manure, wood shavings, grass, hay, and straw—all maintained uniformly at the depth of 1 inch.

It is seen from Table II that so far as the conservation of moisture is concerned, straw is by far the most effective, and then come hay, grass, wood shavings, and manure, in the order of their effectiveness. The difference between the first and the last is 54.1 per cent; hence the importance in choosing the proper type of mulch material. Such a profound difference demands an elucidation. It was observed that the straw was the most loose and open, whereas the manure mulch formed a uniform and compact covering. When the experiment was over, the mulches were removed and their moisture content determined (Table III).

TABLE II.—*Loss of moisture during 33-day test*

Can No.	Manure.	Wood shavings.	Grass.	Hay.	Straw.
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1	1,004.4	825.0	679.5	674.1	480.4
2	980.7	782.6	665.2	661.1	439.7
3	980.5	772.8	668.6	656.3	437.9
Average loss with manure as 100 per cent	987.5	793.5	670.4	663.8	452.7

TABLE III.—*Percentage of moisture content of mulches*

Manure.	Shavings.	Grass.	Hay.	Straw.
144.7	23.2	22.7	20	11.8

A comparison of Table III with the foregoing will show that there is a correlation between the moisture lost from the soil and the moisture contained in the mulches. The moisture content of the mulches varies from 144.7 per cent for the manure to 11.8 per cent for the straw, and the total moisture lost from the soil varies accordingly. That is to say, the more absorptive the mulch the more moisture is lost from the soil. Since the purpose of a mulch is to check the soil surface from exposure to the physical agencies of evaporation, the less absorptive the mulch the less water is brought up to the surface to be exposed and therefore the more moisture is held in the soil. Due to the large amount of colloid matter present in the manure, it is capable of absorbing water one and one-half times its own weight, while the dry straw absorbs only one-tenth of its weight. With different quantities of water distributed in approximately equal volumes of manure and straw, it stands to reason that there should be more exposed by the former than by the latter. The same principle can be applied to the intermediate mulches.

However, the preceding tables do not show a reasonably close correlation between the moisture lost from the soil and the moisture content of the mulches, which tends to show that there must be some other factor or factors which exercise a certain degree of influence upon the effectiveness of the different mulch materials. That the effectiveness of mulch materials is materially influenced by their capillary system is proved conclusively by a similar set of mulches suspended in wire gauze. The mulches, otherwise the same, were suspended about ¼ inch above the soil surface, so that the direct capillary system between the soil and the mulches was severed. The results are shown in Table IV.

TABLE IV.—*Loss of moisture through mulches in contact and mulches in suspension, illustrating the action of capillarity*

	Shavings.	Hay.	Grass.	Manure.	Straw.
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
Mulches in contact	793.5	663.8	670.4	987.5	452.7
Mulches in suspension	431.5	392.2	471.2	785.2	387.8
Difference	362.0	271.6	199.2	202.3	64.9
Percentage of loss due to absorption	53.6	59.1	70.3	79.5	83.7
Percentage of loss due to capillarity	46.4	40.9	29.7	20.5	16.3

The word "absorption" as used in Table IV is construed to mean more than the retentive power of the mulches, but includes any water that has escaped through the mulches other than by the capillary action. The difference is brought about by the severence of the capillary system between the soil surface and the mulches, or, in other words, it is the total moisture lost minus that which is evaporated through the capillary action of the mulches. It may be noted that in every case there is more

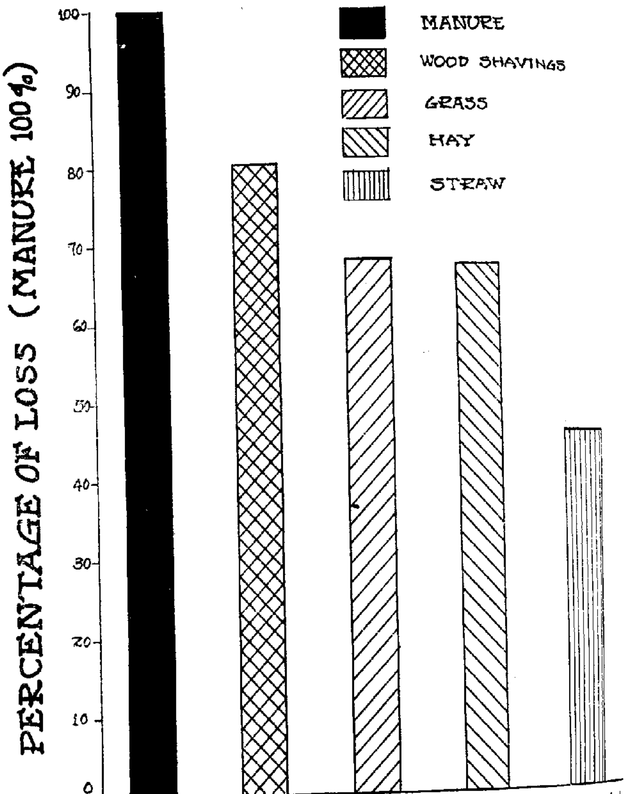

FIG. 1.—Diagram showing the relative losses of moisture from sand with the application of different kinds of mulch materials.

loss due to absorption than to capillarity, although with the wood shavings the difference is trifling. From what has been said, the general rule may be laid down safely that an effective mulch must be constituted of that kind of a material which does not absorb or retain moisture readily and which forms practically no capillary system in itself. A mulch material like manure, which is very porous and therefore holds a good deal of moisture, should not be allowed to stand on the soil except in wet seasons, but must be turned under and thoroughly mixed with the soil before the dry season starts in, otherwise excessive evaporation is likely to take place and the fertility of the manure will deteriorate.

DEPTH OF MULCHING

The depth of mulching has just as much bearing upon the soil moisture as the mulch material. Experiments were carried out with straw and sawdust in galvanized tubes, 36 inches tall, all having the same diameter of 3.5 inches. The bottom of the tubes was perforated to allow free passage of water with the aid of the capillary action of sand. The depths tested were 1 inch, 2, 3, and 4 inches. Water measurements were taken on May 31 and were followed up at various intervals until June 28.

TABLE V.—*Loss of moisture during 60 days with different depths of mulches*

Depth.	Sawdust.	Straw.
Inches.	*Cc.*	*Cc.*
1	309	250
2	173	162
3	110	112
4	104	109

During the same period the unmulched or bare sand lost 531 cc. of water. This will serve to illustrate the point that the efficiency of a mulch increases with its depth. In both cases more water is saved when the depth is increased. Between sawdust and straw, when the depth is beyond 2 inches, there does not seem to be any material difference in the amount of moisture lost, but with the 2-inch and especially the 1-inch mulches, straw is more efficient.

MULCH ACTIVITY UPON DIFFERENT SOIL CLASSES

As the soil particles diminish in size, and therefore increase in surface, more water is brought to the surface. Soils that contain any appreciable amount of porous bodies, hydrates, or colloid matter have a larger water-holding capacity. Since the rate of evaporation is proportional to the moisture content and the extent of exposure of the soil particles, different classes of soil naturally will evaporate variable quantities of moisture.

This subject in question is studied in connection with mulches. Duplicate samples of sand, loam, and clay were mulched with 1 inch of hay.

TABLE VI.—*Loss of moisture during 55 days from different classes of soils under 1-inch straw mulch*

Sand.	Clay.	Loam.
Cc.	*Cc.*	*Cc.*
736	759	846

The same principle seems to govern the evaporation ratio of these three classes of soils. Sand, being much coarser than either clay or loam, lost the least quantity of water. Loam lost the most moisture because its physical structure is much finer and yet passage of water is not

interfered with. Clay has the finest particles, and accordingly it might have been expected to lose the most moisture. But the clay used in this experiment had been ground into powder in the laboratory, and the particles were so fine that the freedom of the movement of water was retarded and therefore evaporation was checked.

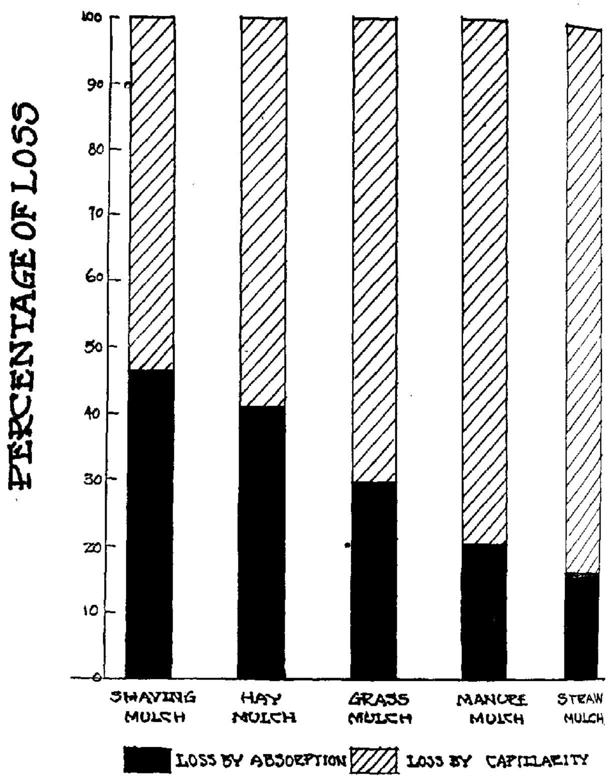

FIG. 2.—Diagram showing the loss of moisture due to absorption and capillarity, taking the total loss as 100 per cent.

EVAPO-TRANSPIRATION RATIO AS AFFECTED BY MULCHING AND CULTIVATION

In so far as mulching or cultivation has the power of preserving moisture in the soil, what effect do they have upon the transpiration of plants? Do the plants make use of the water economically with mulching or cultivation?

The apparatus designed for this experiment consisted of a soil can with a water tank connected at the bottom. Water from the tank was allowed to run into the soil can, and it in turn was brought up to the surface by the capillary action of the soil. Measurements of the water surface were made twice a week by means of an evaporation gage

micrometer. The soil surface had an area of 7.07 square inches through which moisture could be lost.

Six seeds of Long White Icicle radishes were planted in each set of apparatus, of which four germinated in the can marked "no mulch" and "cultivation twice a week," and five in the can marked "cultivation once per week" and "1-inch straw mulch." Seeds were sown on June 23, and the experiment was terminated on July 23. Cultivation was performed by scarifying the surface inch of soil.

The saving of water is a little over 2 inches greater with cultivation than without, and the effectiveness of cultivation in preserving soil moisture increases with the extent of the cultivation. The 1-inch straw mulch saves even more moisture than does cultivation. The effect of cultivation and mulching upon the growth of the radishes needs con-

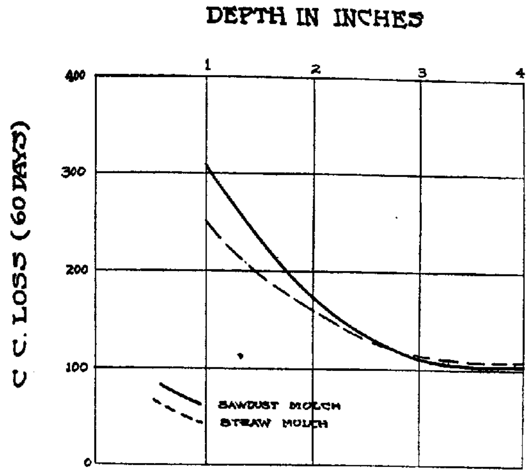

FIG. 3.—Diagram showing the efficiency of mulches with the increase in their depths.

sideration. Without mulching or cultivation radishes are vigorous and the roots uniform, while with cultivation once or twice a week the plants are not so thrifty and the growth of roots is inclined to be stunted. With the 1-inch straw mulch the growth is medium, but the roots are slighlty shorter and smaller than those grown without mulching. The water cost of one part of radishes on the dry-weight basis is, without cultivation or mulching, 1,009; with 1-inch straw mulch, 1,045; with cultivation once a week, 1,422; and with cultivation twice a week, 2,262. In other words, cultivation and mulching save more moisture, but without cultivation the plants make the most thrifty growth.

So far as the conservation of moisture is concerned there is no question that cultivation and mulching are beneficial. The fact that with cultivation and mulching the plants do not grow vigorously is due to three unfavorable conditions.

First, there is too much water within the root zone, the water table being only 15 inches below the surface. The water has been accumulated to such an extent that it is far beyond the optimum moisture content of

the soil. This, indubitably, has a tendency to shut off the air from the roots, which is essential to their proper growth. As it is, they can not penetrate deeply, which seems to be the main causes for their growth's being stunted.

Secondly, cultivation cuts off the root hairs; thus the normal function of the tap root is much handicapped. Frequent cultivation, once or twice a week, does not give the tap root enough time to build up its new root hairs.

Thirdly, frequent cultivation renders the soil too loose, thereby breaking up the contact of the roots with the soil. Since the difference between the soil surface and water is only 15 inches, and the soil, being partially saturated, is already loose enough to induce a favorable root expansion constant stirring of the soil simply destroys the necessary degree of compactness around the root.

Under field conditions in the dry-farming regions where the precipitation is not over 20 inches annually, there is no chance of ever saturating the soil for any length of time. Cultivation or mulching can be recommended only where water becomes the limiting factor to crop growth. If there is enough precipitation to warrant a good and vigorous growth, or if the water table is near the surface, cultivation or mulching may be entirely out of place.

TABLE VII.—*Evapo-transpiration ratio of radishes as affected by mulching and cultivation*

Treatment.	Evapo-transpiration.					Weight of radishes.			Evapo-transpiration ratio (parts of water to 1 part of radishes by weight).
	Germination stage.	Seedling stage.	Root stage.	Total.		Tops.	Roots.	Total.	
				Inches.	Kgm. equivalent.				
	Inches.	*Inches.*	*Inches.*			*Kgm.*	*Kgm.*	*Kgm.*	
No mulch	3.629	4.212	5.292	13.133	99.349	0.0251	0.0733	0.0984	1,009
Cultivation once a week	3.526	3.654	3.895	11.075	83.777	.0205	.0384	.0589	1,422
Cultivation twice a week	2.771	3.623	3.415	9.809	74.207	.0160	.0168	.0328	2,262
1-inch straw mulch	2.538	2.988	3.793	9.319	70.526	.0187	.0488	.0675	1,045

SOIL MOISTURE AS AFFECTED BY TIME OF CULTIVATION

The question of spring or fall plowing has been one of the dry-farming problems. Opinions seem to differ. An experiment was carried out on the Nephi substation on $\frac{1}{10}$-acre plots to determine the moisture content of the soil by spring and fall plowing and by the different depths of cultivation.

TABLE VIII.—*Average soil moisture percentages to the depth of 6 feet with weeds pulled and the different depths of cultivation under spring and fall plowing*

	Spring plowing.	Fall plowing.
Weeds pulled	18.7	20.[illegible]
2-inch cultivation	19.4	20.[illegible]
4-inch cultivation	20.4	21.[illegible]
6-inch cultivation	19.4	20.[illegible]

Moisture samples taken from the Nephi substation to the depth of 6 feet prove in every case that fall plowing preserves more moisture than spring plowing. The 4-inch cultivation is more efficient than the 2-inch, but the 6-inch cultivation drops off a little. The table also shows that cultivation is better than clean culture.

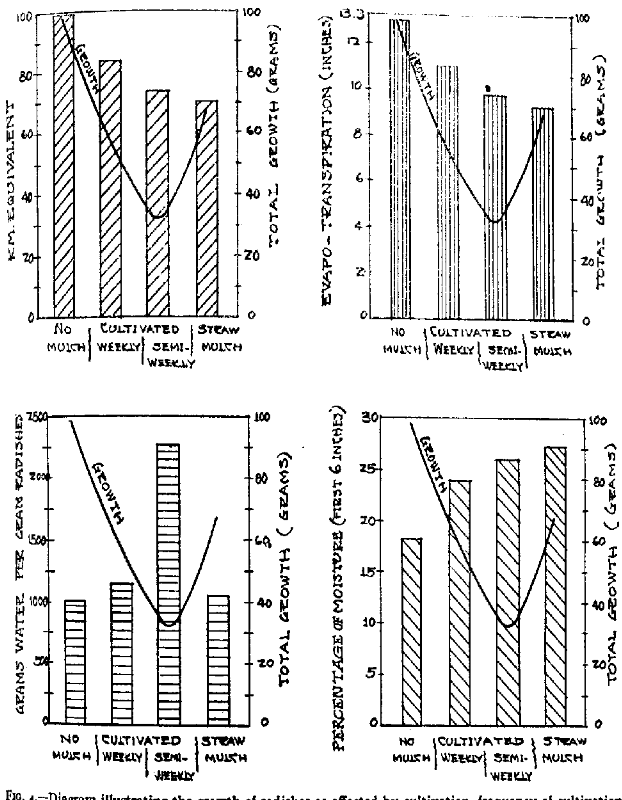

FIG. 4.—Diagram illustrating the growth of radishes as affected by cultivation, frequency of cultivation, straw mulch maintained at the depth of 1 inch, or no treatment, and also the percentage of water in the surface 6 inches of the soil.

MOVEMENT OF SOIL MOISTURE UNDER MULCHES

When soils of different moisture contents come into contact there is a movement of moisture from the thick films of water around the soil particles to the thin films, until equilibrium is reached. That is, water tends to move from the wet to the dry soils, and the influence of cultivation can be proved indirectly by tracing the direction in which the soil moisture moves. At the Nephi substation, $\frac{1}{10}$-acre plots were set aside for mulch experiments. The adjoining strip of land was neither mulched nor cultivated. Four borings to the depth of 6 feet were taken 23 feet apart, from the middle of the plots to the adjoining unmulched land.

TABLE IX.—*Average moisture percentages to the depth of 6 feet, showing the movement of water under mulches*

	Middle of plot.	23 feet from middle.	46 feet from middle.	Unmulched land.
Fall-plowed, straw mulch 4 inches	24.7	23.0	21.7	21.
Fall-plowed, earth mulch 4 inches	21.4	21.4	21.2	21.
Spring-plowed, earth mulch 4 inches	21.4	21.4	19.4	19.

The highest moisture percentages are found in the middle of the plots. Moisture percentages of the soil decrease as they approach the unmulched land, and the lowest are found in the unmulched land. If there is no move-

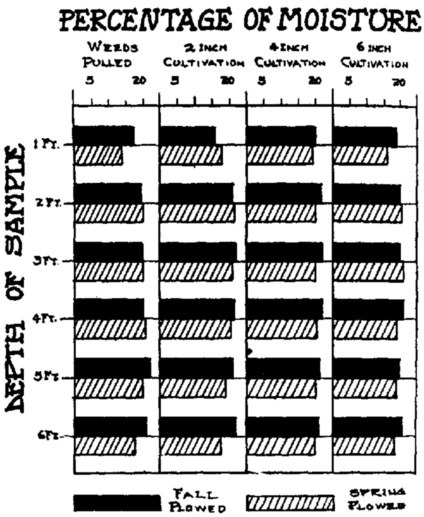

FIG. 5.—Diagram showing the retention of soil moisture as influenced by clean culture and the different depths of cultivation to the depth of 6 feet in the fall-plowed and spring-plowed plots.

ment of water, then there should be the same amount of water anywhere under a uniform mulch. The fact that there is more moisture in the middle of the plots and less moisture as it approaches the adjoining unmulched land shows plainly that water moves out from the mulched plots to the unmulched land. Under laboratory conditions, where side leaching and deep percolation of water can be controlled, the effectiveness of mulching can be determined easily. But under the field conditions, the same factors can not be controlled. Whatever water may be saved by mulching may be lost through either of the foregoing processes. Thus it is not uncommon to find that a mulched plot may not have any more moisture than the unmulched, or that a thicker mulch may not be so effective as a thinner one. As shown in Table VIII, the 6-inch cultivation, either spring-plowed or fall-plowed, is not so efficient as the 4-inch. It is very probable that when the mulch is kept too thick, light rainfalls

can not wet it thoroughly, and since its capillary system with the subsoil is not connected up, no water can be drawn downward. The water is being held in the upper layers of the mulch and then lost to the air

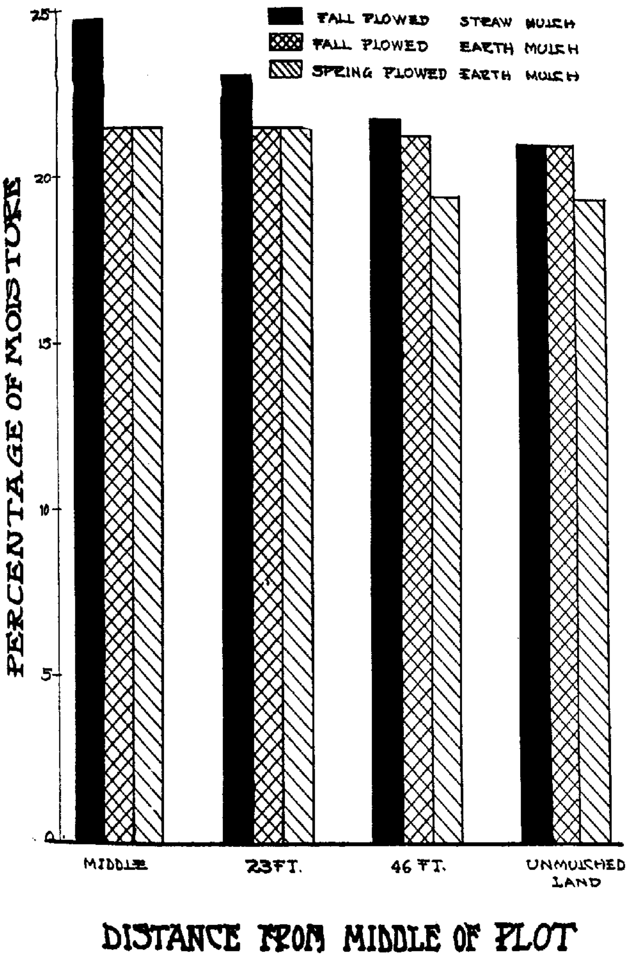

FIG. 6.—Diagram showing the gradual decreases of moisture in the soil to the depth of 6 feet from the mulched plots to the adjoining unmulched land to illustrate side leaching.

directly and completely. At Nephi, or elsewhere in the dry-farming regions, where the annual precipitation is low and only a small part of it comes during the summer months, shallower cultivation is to be preferred to the deeper.

SUMMARY

(1) The question of the effectiveness of mulching has been much disputed. Further study is desirable. The results of these experiments are reported in terms of averages from thousands of measurements.

(2) An effective mulch of 1 inch of straw is capable of preserving 60 per cent more moisture in the soil than is retained without mulching.

(3) Straw is the most efficient mulch material used in the experiments. Then come hay, grass, wood shavings, and manure, in the order mentioned.

(4) The loss of moisture from the soil is correlated with the percentage of moisture retained by the mulch. An efficient mulch must be constituted of material which does not absorb or retain moisture readily and which forms practically no capillary system in itself. An absorptive mulch can be used to an advantage provided it is turned under before the dry period begins.

(5) The effectiveness of mulching and cultivation increases with their depths.

(6) The rate of evaporation of soils under mulch varies according to their moisture contents. Finer soils lose more water. Clay that has been ground into a fine powder in the laboratory loses less water than loam.

(7) Cultivation and mulching save more moisture, but the evapo-transpiration ratio is the least with no mulch or cultivation.

(8) Fall plowing preserves more moisture than spring plowing.

(9) Under field conditions, soil moisture is more variable. The 6-inch cultivation is not so efficient as the 4-inch. In the dry-farm regions, shallower cultivation is to be preferred to the deeper.

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ORIGIN OF THE CENTRAL AND OSTIOLAR CAVITIES IN PYCNIDIA OF CERTAIN FUNGOUS PARASITES OF FRUITS[1]

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SCOPE AND PURPOSE OF THE INVESTIGATION

An investigation of fungi associated with rotting strawberries and dewberries during shipment and marketing has afforded an opportunity for study of the development of the pycnidial form of several species of fungi, from which it appears that in these forms there are three principal stages or periods of growth. The first is one of rapid cell multiplication whereby a certain amount of fundamental or undifferentiated tissue is formed with which to begin the construction of the fruit body. The second or intermediate stage includes those activities leading to the formation of the central cavity, the organization of the wall, the delimitation of sporogenous tissue, and, in many forms, the construction of the ostiole. Previous study has largely been confined to the third stage, which includes spore production and other developments which characterize the mature pycnidium. The methods by which the plectenchymatous primordium is formed are also fairly well understood, but just how the characteristic pycnidium is evolved out of the mass of undifferentiated tissue has not been clearly explained. The intermediate stages in the growth of phycnidia of several species have been followed by the writer, and the results of the studies on three of these are reported in this paper.

Bauke's contribution (*2*)[2] is noteworthy because of the clearness with which he describes the two different methods by which pycnidia originate; and on this point, which was his main contribution, his work has been repeatedly confirmed. From the very nature of the subject of his investigation, the method which he employed was not adequate to give the best results. He found it difficult to learn what takes place in the pycnidial "knot" when the cavity is being formed and the sporiferous layer organized, as he was compelled to study the fungus as it grew on slides. In the pycnidium of *Cucurbitaria elongata* a cell at the center becomes divided into a few radially arranged pyramidal cells with their apices together at the center. These are the sporophores or their mother cells. They divide again in various directions or at once cut off spores. By the gelatinization of their walls, the sporophores are separated from each other to form the cavity, which is further increased by the swelling of the mucilage derived from the walls of the spores. In the pycnidium of a Diplodia on Cornus there is at the end of the first period of growth a

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pseudoparenchymatous structure of thin-walled cells bordered by a cortical region. Now at various points in the central portion there appear elliptical areas marked by inwardly growing hyphæ. There are a number of these regions of growth corresponding to the number of locules which will appear in the young pycnidium; some of them may become united as the phycnidium ages. According to Bauke it is the head-on thrust of these young sporophores against each other and the pressure due to the gelatinization of their walls that accounts for cavity formation by forcing the sporogenous layer farther and farther out, crushing the tissue between it and the cortex so that the protoplasm of this crushed tissue can be used in nourishing the spores.

De Bary (*3*), without referring to Bauke's account, describes the development of the pycnidium of a species of Pleospora and says that the central cavity is due to the failure of the cells at the center of the pycnidial primordium to keep pace in their growth with those in the periphery so that the cells at the center are torn asunder. Such a cavity would be schizogenetic.

Baccarini (*5, p. 69*) describes the formation of a cavity in *Sphaeropsis malorum* and adds a further note on the development of pycnidia in general (*6, p. 150–151*). Certain cells at the center of the pseudoparenchyma can be distinguished from the rest by their abundance of oil and glycogen. Their walls become transformed into mucilage which, absorbing water, swells to form a central, oval, or spherical cavity lined with sporophore fundaments. This sporiferous layer plays an important part in the further development of the pycnidium. Baccarini does not say that the entire contents of the cells at the center disappear as the cavity is formed. His account is very similar to that given by Bauke, adding nothing except to state (*6, p. 150*) that lysigenetic and schizogenetic factors enter into the processes, depending upon the quantity of tissue present at the center of the primordium and on the rapidity with which it is reabsorbed.

Reddick (*8*) studied the development of the pycnidium of blackrot of the grape. He states that the activity at the center of the "gnarl" is evidenced by the more deeply staining contents of the cells. He finds that a number of hyphae grow inwardly and assumes that these are the young sporophores. He realizes that the cavity later increases in size, and he believes that it does so in some way at the expense of the pseudoparenchyma. In his description of the origin and the development of the perithecia the nature of the "sclerotium" is discussed. He believes that such a body develops into a perithecium and gives it the name pycnosclerotium.

Hesler (*10*) describes briefly the development of the pycnidium of *Sphaeropsis malorum*. His account is similar to that given by Baccarini, to which he refers. Hesler, however, states without further comment or figures that the cavity is formed by the breaking down of cells and that the ostiole is formed in the same way. He is of the opinion that the dome-shaped tissue (often shown in figures of this pycnidium) from which the sporophores arise is due to irregularities in the cavity which is tending to become multilocular.

In order to best illustrate the relative importance of the two factors disorganization and growth inequalities, in cavity formation, the intermediate stages in three pycnidia will be described. The formation of the ostiole will be considered in connection with one of the forms.

BLACKROT FUNGUS OF DEWBERRY

There is a blackrot fungus of dewberries the pycnidia of which are very similar to those of *Phyllosticta paviae*, *P. labruscae*, and *P. solitaria*. Pycnidia frequently develop on berries from North Carolina found in the New York and Washington markets. It requires but a short time for the black rot to make its appearance if berries are placed in damp chambers. On account of the slowness with which the disease develops in transit and market it will probably not prove of much economic importance.

The fungus has not been connected with its perfect stage, but it may very well belong to *Physalospora carpogena* Atkinson, authentic specimens of which apparently are not in existence. The pycnidial stage has therefore been called by Shear *Phyllostictina carpogena*, and a formal description is to be published.

The fungus has been isolated, and pycnidia have been grown to maturity on agar media. Dewberries artificially infected with spores from these cultures have developed the typical blackrot. An abundance of material from natural sources and from these cultures has been available for study.

FORMATION OF THE CENTRAL CAVITY IN THE PYCNIDIUM

The "gnarl of hyphae" develops into a plectenchymatous body which has been called a "pycnosclerotium" (Pl. 1, A), such as has been noted and described many times by those who have studied the blackrot of the grape. The large outer cells appear dark in color with thick walls, but toward the center the cell walls are very thin. The central portion possesses sufficient food for future structural growth processes. While in this fungus, as well as in that of grape blackrot, it is undoubtedly true that similar bodies are immature spermogonia, or young perithecia, there can be no question that every pycnidium of both species passes through some such form in its development. If cavity formation for some very definite reason begins early, while the primordium is still plastic, then the resemblance to a "sclerotium" is not so marked. These bodies are frequently as large as mature pycnidia. If in such case there is little further enlargement, how may we account for the central cavity of the pycnidium? In the sections shown in Plate 2, A, B, and Plate 1, B, there is evidence of the beginning of disorganization of cells at the center. As soon as one or two cells disintegrate sufficiently, the surrounding ones push into the mucilaginous matter and in their turn begin to disorganize. It may be that degeneration is at first due to the head-on thrust of cells pushing inwardly; but, if one may judge by what follows, it is more likely that the breaking down process begins first.

The cells of the next succeeding layer to push into the small open space present the appearance of rather thick sporophores, which is misleading (Pl. 2, C, D; 1, C), since ordinarily these also become disorganized by degrees, and the cells below them push out in their turn. The wall layers are not as yet well organized. The conditions in this stage are shown in Figure 1, *a*. The cells lining the cavity clearly result from the budding or division of the cells of the original pseudoparenchyma. They do not show in this section very decided effects of pressure from within, such as Bauke (2) says results in crushing and flattening the inner-wall cells. Later, as the cells elongate or even divide tangentially, this

appearance is more exaggerated. From each of these cells two or three hyphae now branch out, each composed of one to several cells, the outermost being in various stages of disorganization. The nuclei are quite distinct; frequently there are two lying close together in each cell. So similarly do the cavities in pycnidia and spermogonia originate that one can not be certain into which such a structure will eventually develop. These protosporophores occasionally produce spores, since a few pycnospores sometimes are found in sections of such young pycnidia, just as spores are rarely budded off directly from the thin-walled cells at the

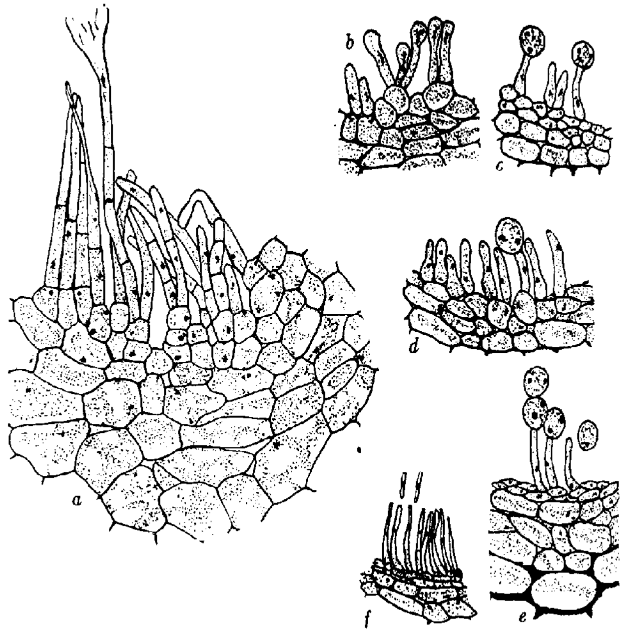

FIG. 1.—Development of sporophores: a, Hyphalike protosporophores growing out from cells of wall which is as yet not definitely organized, showing ends of hyphae undergoing disorganization; b, the first sporophores, sometimes several budding from a single cell, with ends of sporophores swelling in first stages of spore formation; c, sporogenous layer now definitely organized; d, young sporophores, each with a single nucleus; e, sporophores from a mature pycnidium, each spore binucleated; f, spermatiophores from a mature spermogonium.
All drawings made with the aid of a camera lucida Zeiss No. 8 ocular, 3-mm. oil-immersion lens.

very beginning of cavity formation. This may account for the pycnospores sometimes found in spermogonia, to be noted later.

The ostiolar portion has not been developed in the particular pycnidium shown in Plate 1, C; central cavity formation began very early, while the primordium was still little "carbonized" and therefore in a plastic state. Sections often show a large central cavity filled with pieces of the inwardly growing hyphae in all stages of disintegration. New buds to form sporophores arise from the subadjacent cells, which as the result of radial and tangential divisions become much smaller than those which originally occupied this space in the pycnosclerotium. A still older stage is shown

in Plate I, D, where there are no traces of the hyphalike branches; but the big cavity is filled with the stainable disorganized remains, suggesting very strongly that much of the nutrient for spore formation is obtained from within. In more mature pycnidia the central and ostiolar cavities will contain many spores, but no stainable cell remains (Pl. 1, E).

Sporophores are less easily distinguished in mature pycnidia because they are used up more and more as the food supply becomes exhausted in spore formation. This was pointed out by Bauke (2). It would be difficult to prove in any case, but it is not improbable that the entire layer of fertile cells bearing the first functioning sporophores may disappear and new sporophores, one by one, grow out from the cells next in line. The cavity certainly increases in size, and the extent of the tissue lining it diminishes. Plate 1, D and E, shows an elliptical cavity in the necklike portion which serves as a temporary storage space for spores.

SPERMOGONIA OF THE FUNGUS

One finds statements regarding the transformation of spermogonia into pycnidia, or vice versa, or of either of these forms into perithecia. No one has given us the story of what happens to the pycnidia covering a particular grape placed out of doors throughout the winter. After a somewhat extended study of this fungus, the writer is still unable to distinguish positively the bodies out of which spermogonia or pycnidia will be formed. He suspects that each of these spore-producing bodies arises from its own peculiar primordium, notwithstanding the fact that it is not at all rare to find pycnospores in spermogonia.

As noted previously (p. 745), the perfect stage of *Phyllostictina carpogena* remains in doubt. Further evidence that it may be a Guignardia is that it has a spermogonium very much like that of *Guignardia bidwellii*.

Spermogonia and pycnidia may be found side by side on the dewberry, and were it not for their contents these fruit bodies could not be distinguished. In size, shape, and wall structure they may be identical. In many spermogonia there is found the same peculiar elliptical ostiolar cavity (Pl. 1, F) so characteristic of pycnidia of this species. The writer has studied much more extensively the formation of the cavity in spermogonia of *Guignardia bidwellii*. The processes appear to be the same in both species.

CAVITY FORMATION IN THE SPERMOGONIUM

Cavity formation begins rather in the apical portion of the primordium of fundamental tissue where by partial disorganization of cells way is made for growth of orientation by which chains of cells become directed toward the apex. This stage, once initiated, is carried on very rapidly. Protospermatiophores which are long and threadlike soon fill the central portion (Pl. 1, F), each hypha (?) consisting of a chain of rodlike cells surrounded by a mucilaginous sheath. These cells, freed from each other, could scarcely be distinguished from spermatia; and it is possible they are the first spermatia formed. Later, when definite spermatiophores (Fig. 1, f.) can be clearly made out, the cavity is devoid of such threadlike aggregations, and very distinct rod-shaped spermatia lie around in the cavity. This suggests that the structures shown in Plate 1, F and G, now break up and disorganize, leaving the mucilaginous products still in the cavity, and that definite spermatia will soon be formed.

There are eight pycnospores in the spermogonium, a section of which is shown in Plate 1, G. There is now no evidence of the origin of these spores, which were probably formed at an early stage in cavity developement; and the cells from which they were derived have now disappeared. The writer has not seen sporophores in those spermogonia of *Guignardia bidwellii*, which contain a few pycnospores.

It has been shown above that spermogonia and pycnidia are formed from bodies similar to what Reddick (*8*) calls "pycnosclerotia." In many species the tissue composing the "gnarled" primordium is not at all sclerotized. Pycnidia of *Schizoarme straminea* Shear, for example, arise out of a tissue that can not be distinguished from a stroma (Pl. 4, A). The very irregular tuberculate and massive forms taken by the "pycnosclerotia" of *Phyllostictina carpogena* in cultures on agar very frequently become arrested at this point, but so many have been found in the process of cavity and spore formation as to suggest that these structures are fundamentally sclerotized stromata, out of which, or in connection with which, fruit bodies are formed. Pycnosclerotia then, if we may put it that way, are not designed to be resting stages in the life cycle, not necessarily structures into which the fungus is forced (even if frequently compelled to remain there) by unfavorable environmental conditions. They are the result of a regular and natural course of events by which certain quantities of food and fungous tissue are brought together in preparation for spore formation. Having seen that in this Phyllostictina there is little actual crushing of tissue such as was thought by Bauke (*2*) to be the case, the relative importance of the two factors, disorganization and growth inequality, in cavity formation can be judged also by the little average difference in size between the "pycnosclerotia" and mature pycnidia. Allowing for cases such as were noted previously in which cavity formation begins very early, the central cavity in this species is ordinarily, then, largely lysigenetic. We may next turn to a pycnidium of an entirely different type.

ORIGIN OF THE CENTRAL CAVITY IN THE PYCNIDIUM OF THE STRAWBERRY-ROT FUNGUS

Associated also with the rots of dewberries and strawberries is the fungus, *Sclerotiopsis concava* (Desm.) Shear and Dodge, and its conidial stage is very common on other plants (*12*). The pycnidium is large, shield shaped, and without ostiole. The big spore cavity is surrounded by a heavy brown wall several cells in thickness and at maturity is densely packed with spores (*12*, Pl. 5, Fig. 17).

The intermediate stages in development are best studied from sections of material grown on strawberries or on infected leaves placed in damp chambers. In the younger primordia, hyphae invade the epidermal cells, bursting through the side walls and lifting the cuticularized layers, forming a broad, compact, mound-shaped tissue (Pl. 3, A), most of the outer portion of which consists of hyphae spreading outward from a broad base (Pl. 3, B). The cells at the ends of these hyphae after a succession of divisions become elongated peripherally. Now along a line 5 or 10 cells from the outside border a narrow zone of degeneration can be distinguished; cells are losing their contents and elongating as though under strain (Pl. 3, C). This disorganization occurs in intercalary cells shown at the right in the figure. The line of rupture is not necessarily so evenly curved that it follows the contour of the outer wall

it is more often very irregular, dipping down here and there rather deeply (Pl. 3, D). Superficially the pictures presented certainly suggest that the rupture is caused by forces from within, either that the lower two-thirds of tissue has shrunken while the outer third designed as the future wall has remained rigid or that the latter part has been raised bodily by addition of new cells at the ends of the arch, the rest of the tissue remaining dormant. But the early stages of these large, dome-shaped pycnidia develop in nature beneath the tough and tightly stretched cuticle, for example, of canes of *Rubus* spp., where if the rupture were caused in the manner just suggested, there would occur more or less buckling of the outer wall under such unequal strains as must exist. At such a stage (Pl. 3, D) no buckling occurs, which means that the wall must be supported by the pressure from below, furnished by disorganization of the intercalary cells, as indicated. Mature pycnidia on leaves in dry weather are found collapsed, indicating that the thick arched wall in itself is not strong enough to prevent buckling after the pressure has been withdrawn by the shrinkage of the mucilaginous substance within. A concave pycnidium usually indicates that it will be found full of spores. Figures D and E are from a section of the same pycnidium at slightly different planes. Figure E shows somewhat better that the break occurs in radially growing hyphae, but Figure D suggests that disorganization accompanied by the swelling of cells is responsible for the rupture of the tissue. The five or six large bodies mixed in with the tissue just pulling apart (at the center above) are the remains of what just previously were very minute cells of the parallel hyphae and which have now swollen to many times their original size. Many such cells are visible in the section shown at F and also at H, which is still more highly magnified. Above are large masses of small intercalary cells detached from the hyphae which bore them and are now caught between two regions of disorganization. In the section shown at G, the sporophores are fully formed along the entire base; but the mass above these consists only of rows of disorganizing cells, above which is a clear substance, difficult to stain. In this condition the structure might be mistaken for a mature pycnidium nearly filled with spores. Compare this with the section shown at I, which is from a very small pycnidium in which the first spores have just been formed. The spreading, or nearly parallel, rows of cells forming the upper wall are still distinct. The cavity may now enlarge to some extent, and there is little question that growth at the border accounts for some increase in its size. It is noteworthy that there is always more tissue below the line of rupture in a young pycnidium than there is below the true sporophores in a mature structure (Pl. 3, C, D, and I). The cells that are first forced apart in the destruction of tissue and that remain below are homologues of the sporophores, but as noted they provide the material which by swelling forces the wall farther out, incidentally later furnishing food for the development of spores.

It has been shown that the rupture in the tissue is initiated by the disorganization of certain intercalary cells in oriented hyphae and that this occurs not at a period of shrinkage but while expansion is still going on. Pressure is provided by the swelling of the cells torn loose from the wall above or isolated from the vertically growing hyphae below. This force maintains the even curve of the wall as it lifts the resistant portions

of the epidermis above, and marginal growth contributes to the final enlargement of the cavity. We see, then, that the cavity in *Sclerotiopsis concava* is about equally lysigenetic and schizogenetic, and in this respect represents a type intermediate between *Phyllostictina carpogena*, noted previously, and *Schizoparme straminea*, which will now be considered.

SCHIZOPARME STRAMINEA, A NEW STRAWBERRY DISEASE

Pycnidia of a fungus heretofore unreported on strawberry have been found on this fruit from Norfolk, Va., and in the markets of New York and Washington each spring since 1918. The fungus is not abundant, and it does not cause a destructive rot of the berry. The pycnidia are thickly scattered over the surface bursting through the epidermis at the time the spores are mature (Pl. 5, A). The fungus is distinguished from others on the strawberry by the crown of light-colored tissue which surmounts the pycnidium and surrounds the ostiole. The same species also occurs on dead leaves of strawberry and several other plants. In July, 1920, perithecia were found on strawberry leaves along with these pycnidia, and the connection between the two forms was proved by growth in pure cultures. Pycnidia and perithecia are illustrated in Plates 4, 5 and 6. A formal description of the fungus is to be published by Doctor Shear in an early number of Mycologia.

CULTURE STUDIES

The unicellular pycnospores germinate with one or two germ tubes without showing septation previous to or during germination. On cornmeal or potato agar in Petri dishes the mycelium grows outward unevenly, so that the margin is much scalloped or lobed, (Pl. 5, B). Pycnidia are formed in concentric circles, the first maturing five or six days after inoculation. They are at first colorless and are surrounded by a loose weft of hyphae. Sclerosis begins at the base of the ostiole and spreads around the wall along the outer layer of cells, so that the pycnidium finally becomes nearly black.

Artificial infection of strawberries has been made to obtain material for study. The fungus is able to maintain possession except when species of Rhizopus gain entrance at an early stage.

In Petri-dish cultures there is a tendency toward the grouping of pycnidia, with an especially large one at the center (Pl. 5, D). They are held together by the tissue which crowns each one and which now viewed from above resembles a thin stroma; the fruit bodies are always separate in nature.

The ascospores are small and hyaline and thus are not readily located in poured plates, so that the following method was used to obtain pure cultures. Single ascocarps were crushed out in a drop of water on sterilized slides. The asci still containing spores float out in the water. Small drops of this water were transferred to a 4 per cent corn-meal agar in Petri dishes at marked places. As the drop spread out and was absorbed, single asci were located. The spores germinated in the ascus in a few hours, so that by transferring the entire ascus after germination one was able, very easily, to obtain large numbers of pure ascospore cultures. Over 100 single ascus cultures in test tubes and Petri dishes were made in this way, and in every case characteristic pycnidia were

formed in a few days. No perithecia have been seen in cultures either from ascospores or pycnospores.

FORMATION OF THE CENTRAL CAVITY IN THE PYCNIDIUM

The various stages are best studied in material obtained by artificial infection of strawberry and from cultures on agar media where the early stages are somewhat exaggerated and prolonged, so that the growth story can be followed and timed more easily than from pycnidia obtained on fruit and dead leaves. Sections of the pycnostroma show a rather homogeneous plectenchyma of thin-walled cells without any trace of sclerosis; consequently, as we should expect, the force due to the more rapid growth of the peripheral cells resulting in the rupture of inner cells comes most easily and naturally into play.

When the primordium has attained considerable size, forming a spherical or elliptical mass of tissue 50 to 100 microns in diameter (as it is found on fruit or on leaves in nature it is much less), it has stored in it a quantity of food readily available for the developments which are next to take place. The sporogenous tissue will be located on a dome-shaped structure placed at the bottom of the cavity similar to that of *Coniothyrium diplodiella* described by Istvanffi (7); the curve of the dome will correspond roughly with that of the wall above, with a spore cavity between the two tissues. The region in the plectenchyma where the cavity will first appear can be determined in advance of any line of rupture. There is a preliminary growth and orientation by which rows of vertically placed cells are laid down in a line across the upper central portion of the primordium. The upper cells in these rows stretch out or swell as gelatinization progresses, but the cells below the line of final separation continue to grow upward, forming a palisade-like row of elongated cells (Pl. 4, A). As noted, the cells that first rupture appear to be either swollen longitudinally or stretched. If the rupture were due to the excess growth of cells in the periphery over that of the cells within, this appearance of stretching would be a natural one; but it should be kept in mind that there has been a vertical growth of the hyphae that are being ruptured. The disorganization may thus be due to the thrust of the growing cells into those above, or to enzym action. In any event these elongating cells are the homologues of sporophores, although the ones present at this time will not bear spores. The end cells disorganize and the colloidal remains stretch out as the cavity increases in size (Pl. 5, E). The end cells slough off, new buds put forth, and new potential sporophores arise by division of cells of the enlarging dome of fertile tissue. In material grown on agar there is certaily a large amount of disorganization of tissue of the pycnidial stroma, but in nature this structure is not great, and differentiation of tissues begins very early so that the lysigenetic enlargement of the cavity is less apparent. That there is here a certain amount of cell destruction is evident from the cell remains still clinging to the inner layer of the wall of the mature pycnidium (Pls. 4, J; 6, F). So characteristic is this line of disorganized cells that our fungus was recognized in sections of oak leaves from Italy collected by Professor Massalongo 30 years ago.

DEVELOPMENT OF THE PYCNIDIUM ON STRAWBERRY

On strawberry the pycnidial primordium reaches but little size before it can be seen in sections that the cells just below the peripheral layer at the top are being oriented vertically (Pl. 4, F). There are at first not more than a half dozen rows, two or three cells in each row, in this palisade (Pl. 4, G). These appear, as it were, to be thrust against the periphery, flattening out the cells so that one would say that the wall now consists of only two or three layers of cells, disregarding the buffer tissue which is now burrowing through the host above and which takes no part in the formation of the wall. The vertically oriented hyphae now branch out and their end cells disorganize, the additional pressure due to mucilaginous disorganization tending in all cases to play a still further part in the organization of the inner-wall cells. These may in turn degenerate or, if not, they may divide tangentially or elongate peripherally so that finally the completed wall consists of five or six layers of cells which are two or three times as long as thick. Sclerosis is so long delayed that the pycnidium is capable of continued enlargement.

GROWTH OF THE BUFFER TISSUE AND FORMATION OF THE OSTIOLE

The formation of the ostiole in perithecia of a few species has been described by Füisting (*1*), De Barry (*3*), Miyabe (*4*), and others, and it has perhaps been assumed that the same principles hold in the development of the ostiole of the pycnidium. In some perithecia there is a disorganization of a strip of tissue leading from the hymenial cavity to the outside. Further enlargement of the ostiole takes place schizogenetically.

In nature the pycnidia of *Schizoparme straminea* are deep seated, and the papillate ostiole scarcely reaches the surface. The work of rupturing the epidermis and other host tissues above is performed by a sort of buffer tissue from the outer loose hyphal envelope, the "tissu fendant" of Istvanffi (*7*) or the "Aperturalgewebes" of Füisting (*1*). An opening must be made through the buffer tissue before the spores can be discharged. It will be shown that the ostiolar opening is formed first through the wall, then passage way through the buffer tissue will be made. The sharp papillate beak results from the upward growth of buds from wall cells.

Soon after the formation of the central cavity has begun, as noted above, the cells at the top of the young pycnidial knot multiply rapidly, burrow into the epidermis, and increase in size by swelling to form a cone-shaped tissue, which is very effective in lifting up, pushing aside, or breaking down the host tissues overlying the pycnidium, after which it appears above the surface as a whitish or cream-colored hood (Pl. 5, E), similar to the "tissu fendant" surmounting the pycnidium of *Coniothyrium diplodiella*. The perforation of the buffer tissue and the disorganization of the wall of the pycnidium in the preliminary stages of ostiole formation are two distinct processes, yet they are both closely associated and necessary in the development of the complete apparatus of spore discharge.

The construction of the ostiole begins at a point in the incipient inner-wall tissue above. A few cells next to the central cavity now forming (Pl. 4, B) begin to disorganize and by swelling provide a small space into which adjacent wall cells bud, turning upward (Pl. 4, E). In the meantime the buffer tissue has developed and performed its function. The disorganization of the wall cells progresses so that the ostiolar opening is

made first, after which disorganization spreads in several directions, and it is the outermost cells of the buffer tissue that are last broken down (Pl. 4, D). The swollen cells of which it is composed contain very little cytoplasm; but there are certain regions in which they show signs of degeneration, accompanied by still further swelling, so that the hood becomes split or ruptured in a very interesting fashion, the lines of cleavage radiating from the point below at which the beak of the ostiole is being formed (Pl. 5, D).

The cells of the buffer tissue still possess the power of growing, for as soon as a cavity has been formed in it by disorganization small hyphal branches bud out (Pl. 4, E). These branches are very soon entirely reabsorbed.

By the time a passage way has been opened through the buffer tissue, other short hyphae have begun to appear from the wall cells, converging to form the beak of the ostiole. The cells lining the ostiole are certainly the homologues of periphyses, but in this case they usually disorganize so that in a fully mature fruit body (Pl. 6, B) little trace of them remains. In the meantime the central cavity has become greatly enlarged, and the remnants of broken-down cells may be seen clinging to the inner wall, as noted above (Pl. 4, J). It is interesting to find that in the ascocarp of this fungus (Pl. 5, F–H) the host tissues are broken open by the same sort of buffer apparatus. This is disorganized in the same fashion and penetrated by the ostiole in like manner; the periphyses are, however, more permanent (Pl. 5, J). In the pycnidial cavity there is much more disorganization of tissue when the fungus is grown on agar media where the absence of confining host tissues may be an additional reason for the delay in the differentiation processes.

Other stages in the life history of *Schizoparme straminea* are illustrated in Plate 5. The habit of growth of pycnidia on the fruit of strawberry is brought out in Figure A, which is somewhat magnified. The buffer tissue which has broken through the epidermis of the host is the only part of the pycnidia visible. On agar media in Petri dishes the mycelium appears to break up into fan-shaped lobes, and the pycnidia form more or less in concentric circles (Fig. B). Surface views of pycnidia on agar such as are shown in Figures C and D bring out very strikingly the manner in which the buffer tissue splits open, frequently in the form of a cross.

That the buffer tissue is composed of cells originating from the upper portion of the outer wall of a pycnidium is clear from the sections shown in Figure E. It is often difficult to distinguish perithecia from pycnidia by the appearance of the buffer tissue exposed, although the ascocarps are frequently much larger than pycnidia. Figure F shows a number of perithecia as they appeared on a much decayed leaf of *Rosa* sp., bringing out well the manner of splitting the buffer tissue, which is so characteristic of the tissue above pycnidia also (Figs. C and D). Mature ascocarps collapse when dry. Sections of a young perithecium through the papillate ostiolar portion (Figs. G and H) show that the buffer tissue evidently functions in rupturing the host tissue, although it soon dries up and falls off after bursting through the epidermis (Fig. I). It is clear from Figures I and J that there are no paraphyses between the asci, although falcate periphyses line the ostiolar cavity. A mature ascocarp with fragments of buffer tissue still adhering is shown in Figure K.

We have in this species a case where the sexual and the asexual fruit bodies are very similar in several respects. The walls of both structures are composed of similar tissue, which is carbonized only at maturity. The ostiole in each form is prominent, being more or less papillate. The straw-colored buffer tissue is always present in both forms, splitting open in a characteristic fashion. The pseudoparenchymatous tissue at the center of the ascocarp breaks down, thus forming a central cavity. One need not confuse the two forms, since the perithecia react to the triple stain in a manner quite different from pycnidia.

The very striking morphological parallelism shown in pycnidia and perithecia of this species raises again the question as to what extent this really occurs among other Ascomycetes, or how much dependence can be placed on such features in the search for other spore forms in the life cycle of a given fungus. Orton (9) called attention especially to various cases of similarity between pycnospores or conidia and the ascospores of certain species. While many examples where there is no such resemblance between the types of spores may be pointed out, it must be admitted that it would be a very natural morphological expression of the inherent physiological or metabolic activities, based upon the constitution of the germ plasm. A feature worked out in the course of time for one spore form must of course have a basis in the apparatus of inheritance. It is surprising that these morphological parallelisms are not more frequent in the Ascomycetes, until one considers the particular environmental conditions under which each type of spore or sporocarp performs its work. The spores of *Hainesia lythri* (Desm.) v. Höhnel and *Sclerotiopsis concava* (*12*) are identical, but the sporocarps bearing them have little in common, because the latter form has taken on the overwintering function. On the other hand, the discocarp stage, Pezizella, resembles the Hainesia.

ABNORMAL ORIENTATION OF SPOROGENOUS TISSUE

It is well known that in cultures of some forms of Ascomycetes it is possible, by changing the direction of illumination, to cause pycnidia or ascocarps to be formed in an oblique or an inverted position. In such cases the structure is inverted or changed as a whole. By suitable orientation of cultures of *Schizoparme straminea* with respect to light, gravity, and aeration the fungus can be induced to build sporogenous tissue at the top of the pycnidial cavity so that the sporophores and spores point downward as they are formed, while the buffer tissue and the ostiole develop in the normal fashion, pointing upward. Figure C in Plate 4 shows a stage in the development of the pycnidium just preceding spore formation. The buffer tissue is not quite full grown, but other sections in the series show that an ostiole is just being formed through this tissue at one side of the sporogenous pulvinus so that, had this pycnidium been allowed to mature and then been cut in a plane at right angles to the one shown here, the relative positions of the sporogenous tissue, buffer apparatus, and ostiole, would be the same as shown in Pl. 6, C. In case the fertile tissue had been directly at the center above, there would have been two ostioles formed (Pl. 6, D); this is in no way associated with two locules in the early stages. What bearing can these curious inversions of the sporogenous tissue have on the question of the methods of cavity formation? This is a complicated situation where we have a central cavity formed, sporogenous tissue delimited,

buffer tissue developed through which a passageway is opened, a wall organized, an ostiolar opening through the wall portions broken down, and a beak developed. The fungus must be able to organize a number of varied localized changes and growth processes harmoniously interacting in such a way as to lead to the construction of a fruit body with certain constant diagnostic morphological features. Such a structure, the cavities or openings in which are formed purely schizogenetically, is difficult to conceive.

As *Schizoparme straminea* is found in nature there is nothing to suggest the presence of a stroma, except perhaps the buffer tissue ramifying through the host tissues and bursting forth, crowning the pycnidium. As explained, this tissue is formed after the knot of undifferentiated primordial hyphae has reached considerable size. Only one pycnidium grows from each knot of hyphae. On agar, however, several pycnida may arise from one of these masses of plectenchyma in such a way as to suggest that the line separating such pseduoparenchymatous primordia from true stromata is not to be too sharply drawn. Again, cavity formation may be started at several different points, followed by a merging of the locules, so that the cavity is at first much lobed or irregular; it finally becomes rounded out by further disorganization readjustments. A multilocular pycnidium would be a very simple step in the evolution from such a type.

As noted previously, there is only a relatively small amount of destruction of tissue during the formation of the central cavity. The ring lining the cavity (Pl. 4, J; 6, F) represents practically all of such disorganization that occurs as the fungus grows on species of Rhus or Quercus. Ostiole formation is usually not begun until the central cavity is distinguishable (Pl. 4, B and E), but no set rule seems to be followed. Ordinarily the first spores are matured before the ostiole is completed. Figure B shows a section in which the position of the ostiole is already determined by the disorganization of cells. The section (E) shows the proliferation of cells of the wall of the pycnidium and the enlargement and disorganization of cells of the buffer tissue.

Füisting (*1*) pointed out how difficult it was to find the early stages of perithecia which he was studying because there is nothing to indicate where they are forming until the Aperturalgewebes bursts through the host tissue, and by that time the perithecium is nearly mature. The same difficulty is encountered in connection with both perithecia and pycnidia of *Schizorparme straminea* as the buffer tissue is not visible until after the first steps in cavity formation have been taken.

DISCUSSION

Partly developed pycnidia of the type found in *Phyllostictina carpogena* have been considered by some authors to be merely young or undeveloped perithecia, especially when they are found in cultures. Some fungi develop vegetatively very well under artificial conditions and even reach the first stage in pycnidium formation to the extent of laying down the plectenchymatous primoridum, yet lack the particular stimulus which sets in motion the metabolic activities initiating the intermediate stages of growth which are fundamentally more complicated than the mere piling up of food in the form of undifferentiated tissue of the primordium.

Very often cell destruction is accompanied with much swelling, which may not be without effect in stimulating further action. In *Schizoparme*

straminea there is a characteristic intercalary growth of orientation before any disorganization sets in. When a weak point is once established and an initial lysigenetic cavity formed, then growth readjustments can come into play, as the results of which the structure may increase in size by the elongation of elements already present at the end of the first period of growth. Intercalary growth in the wall is stimulated, and new cells may be added to the exterior.

Bauke found that in the pycnidium of Cucurbitaria the primordium was often as large as the mature fruit body; therefore, to account for the cavity he supposed that the sporiferous layer was pressed outwardly against the intervening parenchyma so that its cells were crushed. The ultimate effect of pressure due to disorganization is certainly a factor to be recognized, but it is very doubtful whether the cells referred to are crushed to the extent claimed. Those just outside the sporiferous layer are frequently small and flat, due to division, and are not the crushed remains of the large cells once at this point in the primordium. This is quite evident, for a study of Bauke (*2, fig. 12 and 13 of Pl. II*), will show that the cells in the tissue said to be crushed must have undergone division in the process, for the ratio of the number of cells in the periphery of the primordium shown in his Figure 12 to the number in the layer beneath the sporiferious layer is as 3 to 2, while in the mature pycnidium shown in his Figure 13, this ratio is as 2 to 3. Bauke (*2*) and Baccarini (*5, 6*) both state that in old pycnidia sporophores disappear entirely, and many pycnidia have been formally described by authors as without sporophores. The writer has shown that the first hyphae to grow into the initial cavity are not necessarily spore bearing, though they are homologues of sporophores. In *Phyllostictina carpogena* the number of sporophore mother cells increases by multiplication of cells, by radial division, or by budding, while in *Sclerotiopsis concava* and *Schizoparme straminea* the sporophores increase by branching or budding of cells in the dome-shaped fertile tissue. Every cell in the thin-walled central region is potentially sporogenous. Sporophores are often sufficiently marked to be diagnostic, but that they are constant or permanent structures is not borne out by the facts learned from a study of these intermediate stages. That the sporogenous tissue by nature is fundamentally different from every other in the primordium and that, once set apart in the early ontogeny, it remains a permanent, unchanging reproductive tissue is an idea probably carried over from zoology.

Whether the contour of the sporogenous tissue is at all determined by the particular method of cavity formation may be very doubtful, but it so happens that the fertile tissue of the Phyllostictina blackrot of dewberry is strongly concave in outline; in *Sclerotiopsis concava* the incipient or potential spore tissue is convex, but by further disorganization and growth readjustments it soon becomes flattened out so that it forms an even layer extending across the base of the broad flat pycnidium; in *Schizoparme straminea* the fertile layer is sharply convex. It has been shown that in the first species the central cavity is largely lysigenetic. The cavity contains now a mass of predigested food readily available for spore formation, and it should be borne in mind that the fungus provides a large amount of food for this work when the fundamental tissue is developed so that in this pycnidium it is not necessary that more should be drawn from the vegetative hyphæ. In *Sclerotiopsis concava* the central cavity is perhaps about equally lysigenetic and schizogenetic. It is formed in much the same way as it is in the deep-seated

aecidia of the rusts [Peridermium, Adams (*11*)], where the gametes are intercalary cells below in a series of sister cells which are becoming disorganized or have been sacrificed in order to make space and to furnish food for spore production. In *Schizoparme straminea* the cavity is argely schizogenetic, although in artificial cultures on agar there is a arge amount of cell destruction.

Some information has been obtained showing that the same factors, isorganization and inequalities in growth, are operative in ostiole formaion. In view of the fact that the natural relative positions of the porogenous tissue and the ostiole can be altered in a very striking manner n cultures of *Schizoparme straminea* it is much less difficult to conceive of a localized weakness in the pycnostroma determined by metabolic hanges leading to disorganization which initiates cavity formation than it is to understand how the two different regions of rupture initiating the central cavity and the ostiole can be provided for so long in advance in a purely mechanical way.

In *Phyllostictina carpogena* there is usually a secondary cavity formed in the necklike extension of the immature pycnidium. This is an elliptical cavity which is finally continued downward into the pycnidium and thus becomes a part of the ostiole. Not infrequently it becomes from the first a superficial accessory pycnidial cavity lined with sporophores which cut off spores. These sporophores soon become gelatinized and an opening is formed connecting the two cavities. Sometimes the ostiolar portion is rather small and the cavity is lined with short periphyses, showing in a very striking manner that sporophores and periphyses of this species are homologous structures, or that periphyses may on occasion cut off spores.

The picture of *Schizoparme straminea* (Pl. 4, E) shows that no sooner has an opening been effected in the pycnidial wall by means of disorganization than the cells about the opening bud out or proliferate rapidly, growing into the space and turning upward, after which some of the cells at the ends of these new hyphæ in their turn become disorganized. What is it that stimulates the comparatively old wall cells to such a rapid renewal of growth? We see that there must be a change in pressure, and a new supply of food readily assimilable is furnished by the destruction of cells next to them. Even the swollen cells of the buffer tissue seemingly almost devoid of cytoplasm are still able to grow once the adjacent cells furnish a little food through disorganization.

SUMMARY

The intermediate stages in the development of the pycnidia of three species of fungi associated with fruit rots have been studied, and in each case it has been shown that the initial stage in cavity formation consists in the disorganization of cells in a certain region, accompanied by a swelling of the cell remains. The pressure thus resulting tends to enlarge the cavity, maintain a symmetrical contour, and contribute further toward cell destruction or change of form. Further enlargement of the cavity may be effected by the growth of elements already present or by the addition of new tissue.

In *Phyllostictina carpogena* the cavity is formed lysigenetically for the most part, although occasionally excessive peripheral growth plays some part in the process, especially if cavity formation begins early or before sclerosis has become far advanced.

In *Sclerotiopsis concava* the central cavity is about equally lysigenetic and schizogenetic. The swelling of certain cells of the more or less parallel hyphae composing the primordium causes a rupture along irregular lines, extending across the pycnidium, above and below, thus cutting out a strip of tissue which soon completely disorganizes. The cavity is further enlarged by the addition of new tissue at the border of the pycnidium. There is no ostiole in this pycnidium; spore discharge is effected through the bursting of the wall due to the swelling resulting from inbibition and the partial or complete destruction of certain spores at the center of the mass filling the pycnidium.

The central cavity in *Schizoparme straminea*, as it grows on strawberry, is largely schizogenetic. A ring of disorganized tissue usually lines the cavity clinging to the wall. A buffer tissue through which a passageway is opened by disorganization develops soon after the primordium has been laid down. The ostiolar cavity is formed as the result of the breaking down of the cells of the wall in the early stages; the adjacent cells bud out and hyphae grow upward, converging to form the papillate ostiolar structure. In the meantime by the rapid destruction of the buffer tissue along lines radiating from a point above the ostiole this caplike structure is split open in a characteristic fashion.

The first hyphae to grow into the central cavity as it is forming are the homologues of sporophores, but they do not necessarily produce spores, since those first appearing usually disorganize, furnishing substances which by swelling may play some part in shaping the tissues of the wall.

Food for spore production is found in the parenchyma at the center of the immature fruit body in the first two forms. The pycnidium of the third species remains plastic for a long time, so that additional food may be easily obtained from the substratum upon which the fungus is growing.

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PLATE 1

Phyllostictina carpogena Shear

A.—Section of a "pycnosclerotium"; outer cells darkly colored; central cells thin-walled.

B.—Similar structure, a little older. An open space has been formed, and into this space the next surrounding cells have grown.

C.—Further increase in size of the central cavity. Disorganizing cell remains lie about in the cavity.

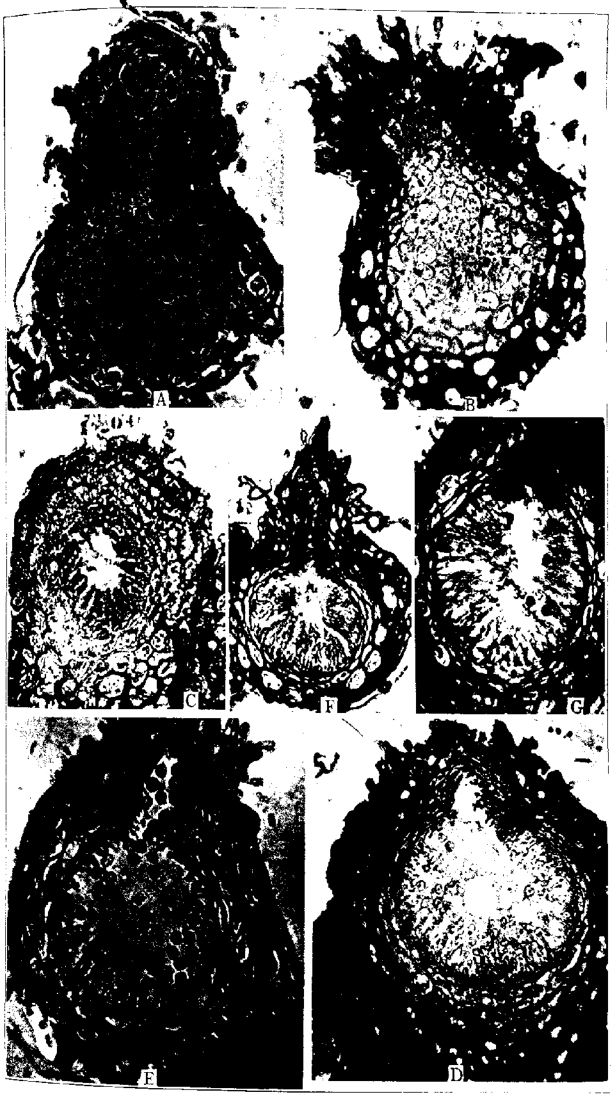
D.—Fully organized pycnidium.

E.—Mature pycnidium, showing a small elliptical ostiolar cavity.

F.—A spermogonium, showing fine, threadlike outgrowths from the inner-wall cells. They have usually disappeared by the time the first distinct spermatia are formed.

G.—An older spermogonium; spermatiophores clearly delimited; pycnospores in the cavity.

Figures B, C, D, E, and G were photographed with a 3-mm. oil-immersion lens; Figures A and F with a 4-mm. dry lens.


A
B
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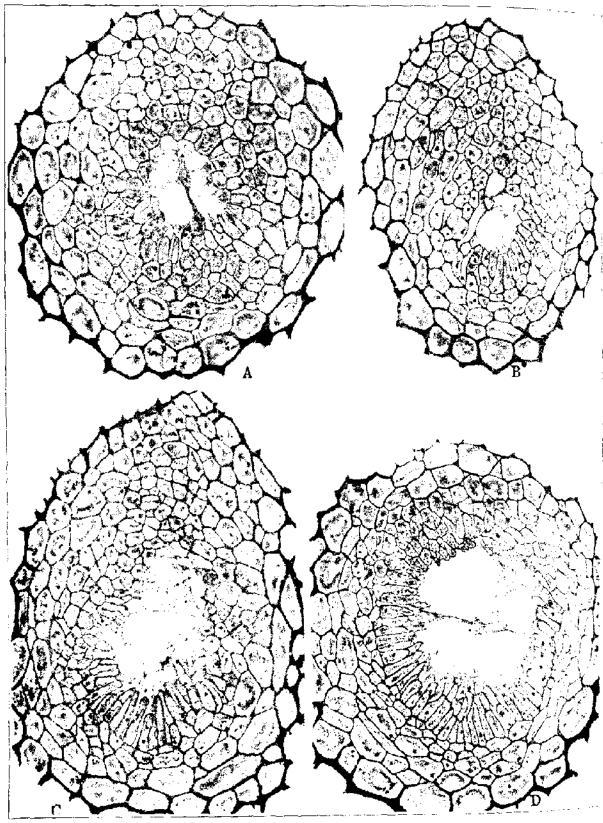
A
B
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D

PLATE 2

Phyllostictina carpogena Shear

A.—Primordium in which a few cells at the center have undergone disorganization; mucilaginous remains of other cells still visible; cells surrounding lower portion of the central cavity beginning to grow inward; two or three layers of "carbonized" tissue surrounding the primordium not shown.

B.—Primordium of a small pycnidium; cavity formation progressing; protosporophores growing inward; "carbonized" portions of outer wall not shown.

C.—Similar to the preceding, but protosporophores more definite.

D.—Layer of cells lining the cavity composed of protosporophores; colloidal cell remains still present in the cavity; inner wall of pycnidium becoming organized; cell divisions in various planes account for the increase in the number of cells.

Figures were drawn with the aid of camera lucida, Zeiss No. 8, ocular, 3-mm. oil-immersion lens.

PLATE 3

Sclerotiopsis concava (Desm.) Shear and Dodge

A.—Mound-shaped mass of hyphae beneath the cuticle; radial growth not evident because this is a somewhat oblique section of the primordium.

B.—Section of a primordium at a slightly later stage, showing plainly hyphae spreading outward from a broad base. The short terminal cells by slight lateral extension take part in the organization of the outer wall.

C.—Still older stage when the line of rupture is well marked. At the right can be seen a small region where the radially growing cell chains have not been ruptured. The break occurs first by the destruction of one or more of the cells of these radially oriented hyphae. The parallel arrangement of hyphal branches below the line of rupture is not the result of an upward growth after the rupture has occurred.

D.—Section of a still older pycnidium, showing quite distinctly five of six swollen cells (center) and other masses of stainable degeneration products in the cavity at the right.

E.—Same section as the preceding except at a slightly deeper focus, showing better how the line of rupture cuts across the radially growing hyphae; degenerating cells at the right.

F.—An older pycnidium, showing many swollen cells.

G.—A pycnidium found on leaf of dewberry; cavity partly filled with masses of detached hyphal cells which originally made up the bulk of the young pycnidial "knot."

H.—More highly magnified picture of a portion of the section shown in F. The very small cells above the large ones are detached portions of the radially placed hyphae, portions of which may become the future sporophores.

I.—Section of a small pycnidium still covered by the tightly stretched cuticle. The first permanent spores formed are still attached to sporophores. There is very little of stainable cell remains in the cavity. The radial arrangement of the cells which make up the wall of the pycnidium can still be plainly recognized.

PLATE 4[a]

Schizoparme straminea Shear

A.—Section at an early stage in the growth of a pycnidium grown on agar; cells above the line of vertically oriented hyphae disorganized, thus determining the line of rupture; no buffer tissue yet formed.

B.—A similar section at a slightly older stage; buffer tissue partly developed; location of the ostiole already determined by some disorganization of tissue; line of splitting below still being extended in both directions.

C.—Section of pycnidium grown on agar under conditions which induce the inversion of the sporogenous-tissue; buffer tissue erect and normal.

D.—Section showing, like succeeding one, that the opening or rupture of the buffer tissue is caused by disorganization and not by a tearing apart due to inequalities in growth.

E.—Disorganization of cells of the hood above and proliferation of cells bordering the cavity; buds growing into the space opened by disorganization.

F.—Young pycnidial primordium on strawberry; large inner cells more or less oriented vertically. Above the center these cells are sending out short hyphal branches which are pushing into disorganizing cells in this region.

G.—Similar to the preceding; protosporophores very distinct.

H.—Section of two young pycnidia grown on strawberry. The buffer apparatus has broken through the host tissue above.

I.—Section of a pycnidium similar to the preceding; gelatinizing ends of the incipient sporophores clearly visible.

J.—Section of a pycnidium found on leaves of sumac; remains of disorganized cells lining the inner wall still attached.

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[a] Figures A, B, and H are from photographs by Marguerite Ickis.

PLATE 5[b]

A.—Pycnidia on a strawberry; only buffer tissue visible.

B.—Characteristic growth of mycelium on agar; pycnidia at first formed in concentric circles.

C.—Pycnidia from similar cultures more highly magnified, showing manner in which buffer tissue splits open frequently in the form of a cross.

D.—In older cultures many pycnidia cluster about the large one first formed at the center.

E.—Buffer tissue seen to be composed of cells originating from the upper portion of the outer wall of the pycnidium.

F.—Large perithecia from a leaf of *Rosa*, sp. They show the same peculiar splitting of the buffer tissue. Mature ascocarps always collapse when dry.

G.—Upper portion of a young perithecium, showing buffer tissue and conical ostiole; opening not yet completed.

H.—Similar to the preceding; opening in the ostiole nearly completed.

I.—Buffer tissue has dried up and broken away. Ascospores just forming; no paraphyses are ever present.

J.—Shows the falcate periphyses lining the ostiole—two branches from each cell.

K.—Mature ascocarp; remains of buffer tissue still present.

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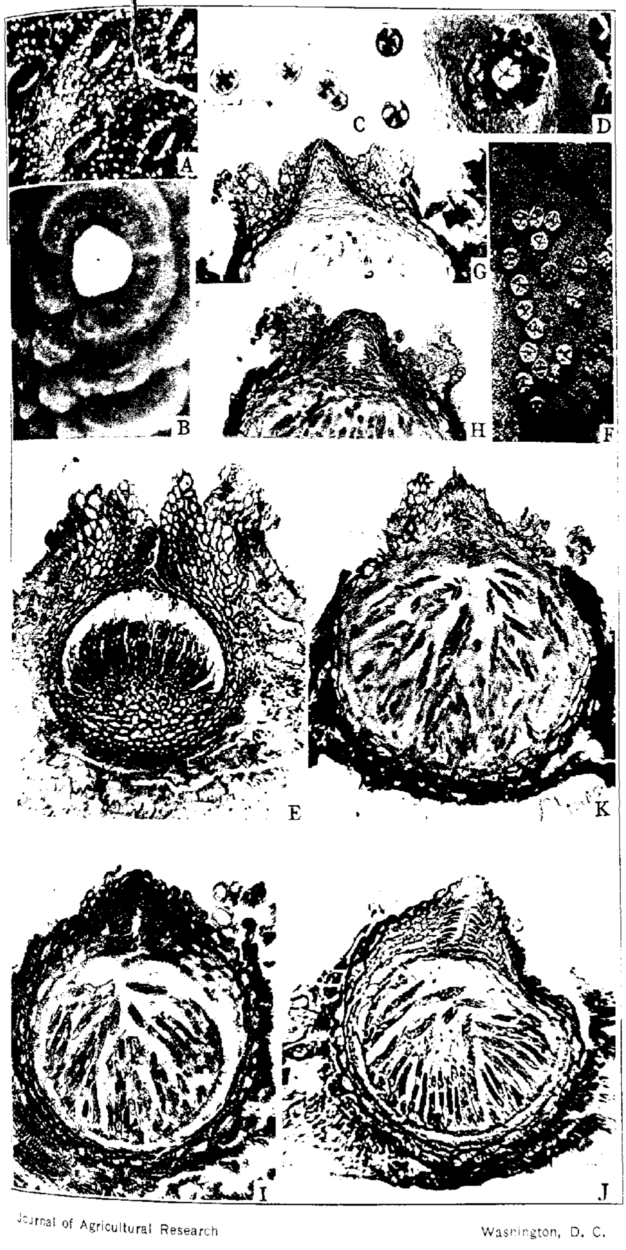
[b] Figures B and C are from photographs by Marguerite Ickis.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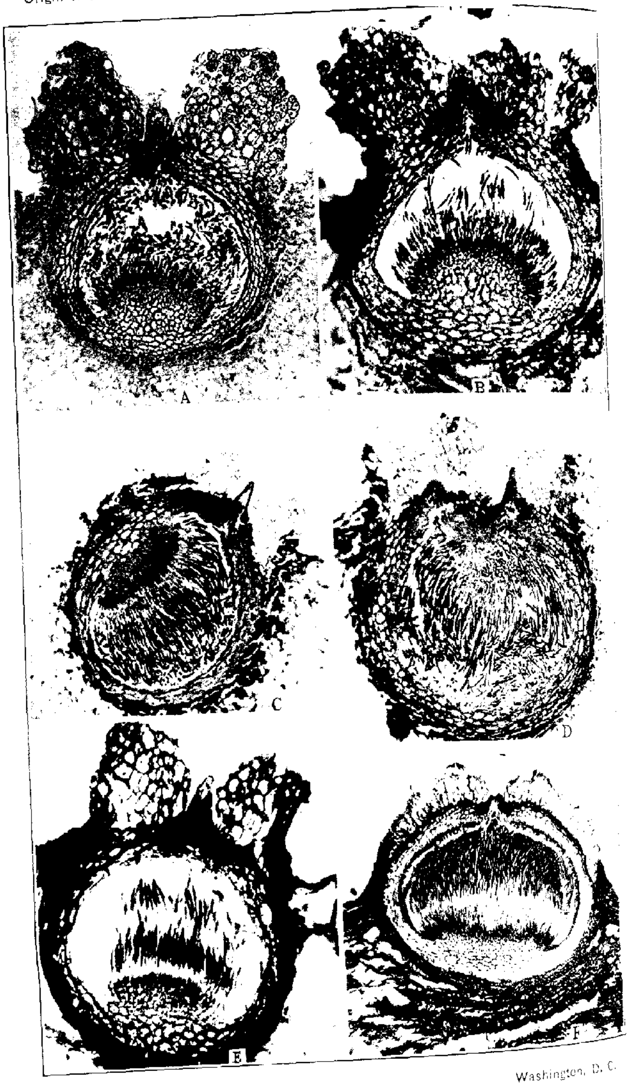

PLATE 6[c]

Schizoparme straminea Shear

A.—Mature pycnidium grown on strawberry; buffer tissue well-developed; walls of cells surrounding the ostiole thickened and blackened; layer of stainable cell remains clinging to inner wall; sporogenous tissue below.

B.—Black conical ostiole more distinct, the only part yet "carbonized."

C.—Mature pycnidium grown on agar in such a way as to cause inversion of the sporogenous tissue; buffer apparatus penetrated by the pointed ostiole oblique to the surface of the medium.

D.—Fertile tissue completely inverted so that two ostioles are formed.

E.—Mature pycnidium grown on strawberry.

F.—Normal mature pycnidium taken from a leaf of sumac; ring of cell remains lining the central cavity.

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[c] Figure A is from a photograph by Marguerite Ickis.

FURTHER STUDIES ON THE PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE[1]

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In 1917 while at the University of Wisconsin the author began a series of studies on the relation of soil temperature as a factor affecting the pathogenicity of *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn). The results from that part of the work conducted with the potato under control conditions in the greenhouse formed the basis of an early publication.[2] During 1918 and 1919 these temperature studies were extended into the field, where the potato plant and the parasite were grown together under natural soil conditions.[3] It is the purpose of this paper to present the result of these field experiments.

In the greenhouse experiments as reported, several crops of potatoes were grown under controlled conditions in tanks at soil temperatures ranging from 9° to 30° C. The results obtained indicate definitely that while the sterile or "Rhizoctonia" stage of *Corticium vagum* may attack and produce lesions on the potato stems throughout this entire range of temperature it becomes seriously parasitic on this host only at soil temperatures below 24°. Injury to the cortex of the young stem was found especially severe throughout a range of from 12° to 21°, reaching its maximum expression at 18°. Deep cortical lesions, however, frequently occurred at a soil temperature as low as 9°. As explained in the previous report,[2] the most serious type of injury was found to occur at the growing tips of the young sprouts, as these were frequently attacked and killed before they reached the surface of the soil. This particular type of tissue destruction was found especially prominent between 12° and 18°. Above 18° such growing-point destruction decreased rapidly and disappeared completely above 21°. The pathogenic power of the parasite in fact appeared to be so definitely inhibited by soil temperatures above 21° as to render the fungus practically unimportant as a pathogen upon the potato above 24°.

It was further demonstrated in these greenhouse experiments that the young potato shoots grew through uninoculated soil most rapidly at the high temperature of 24° C. For continued growth during the later periods of development of the normal plant, 15° to 18° was found most favorable, while at the high soil temperatures of from 24° to 30° the plants were thrown seriously out of balance and exhibited a number of interesting abnormalities. The rapid growth of the shoots at 21° to 24° was found to be an important factor in the escape of the growing points from injury in the inoculated soil.

[1] Accepted for publication Jan. 16, 1922.

[2] Richards, B. L. PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE. *In* Jour. Agr. Research, v. 21, no. 7, p. 459-482, 5 fig., pl. 88-93. 1921. Literature cited, p. 481-482.

[3] The writer again wishes to express his indebtedness to Prof. L. R. Jones, of the University of Wisconsin, for his helpful suggestions and criticism, and to Dr. W. A. Orton, of the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, for his cooperation in the field work conducted at Plainfield, Wis.

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The relative value of these data as an index to the pathogenic action of the fungus under natural conditions of potato culture in Wisconsin was indicated in field studies conducted at Madison and Plainfield.

SOIL INOCULATION WORK AT MADISON, WIS.

During the season of 1919 four successive plantings of Irish Cobbler potatoes were made at approximately 10-day intervals in soil inoculated with the sterile mycelium of *Corticium vagum*. In the process of planting, a pure culture of the fungus grown on a sand-cornmeal medium for four weeks was placed in the soil and two one-half tubers, disinfected before cutting, were planted directly above but in contact with the culture. All sets were then covered to a depth of approximately 6 inches. One plant was grown in uninoculated soil, as a control, for every two plants grown in inoculated soil. In order to obtain an adequate range of soil temperature the first crop was planted at the early date of April 26. The additional successive plantings were made in parallel rows at the various dates of May 7, May 17, and May 30. Observations on the plants in each row were made approximately six weeks after planting. The results of these observations are recorded in Table I. A thermograph record was kept of the soil temperature at a depth of 2 inches[4] for the months of May and June. These temperatures, together with the daily precipitation for the same two months, are recorded in Table II.

TABLE I.—*Effects of Corticium vagum on potato crops planted at various dates through April and May*

Date of planting.	Total hills harvested.	Total stems examined.	Stems slightly injured.		Stems severely injured.		Growing tips destroyed.		Total stems injured.
			Number.	*Per cent.*	*Number.*	*Per cent.*	*Number.*	*Per cent.*	*Per cent.*
Apr. 26	32	182	17	9.3	27	14.8	47	25.8	50.0
May 7	27	202	14	6.9	47	23.2	52	25.5	56.0
17	21	118	19	16.1	14	11.8	8	6.7	34.7
30	25	108	33	30.5	26	24.0	6	5.5	60.0

The result of the experiment shows no specific correlation between the time of planting and the percentage of stems showing injury; on the other hand, the type and intensity of injuries produced by the fungus are evidently very closely related to the date at which plantings were made. As shown in Table I the plants grown from the tubers planted April 26 to May 7 exhibited a high percentage of growing-point destruction as well as a large number of stems which were severely injured. In comparison, the stems grown from the tubers planted on the two later dates of May 17 and May 30 were largely free from growing-point injury and on the whole exhibited lesions considerably less severe than those shown on the plants of the two earlier crops.

A more striking feature of the experiment was noted in the fact that the secondary stems which grew later from the seriously injured primary

[4] Thermograph records of the soil temperature were also kept at a depth of 4 inches for the first two weeks of May. A comparison of the mean daily temperature at the depths of 2 and 4 inches showed an average daily difference for the period of 2.2° C. The daily mean temperatures at 4 inches after May 19 are calculated by subtracting 2.2° from the daily mean at 2 inches. The results are recorded in Table II.

shoots of the first two crops remained practically free from injury (Pl. 1, A, B). The growing tips of these secondary stems showed no indication of injury, while such injury as did occur was very slight and confined entirely to the outer layers of the cortex.

TABLE II.—*Daily soil temperatures and precipitation for Madison, Wis., for the months of May and June, 1919*

Date.	Soil temperature at depth of—				Rainfall.[1]	Date.	Soil temperature at depth of—				Rainfall.[1]
	2 inches.			4 inches.			2 inches.			4 inches.	
	Max.	Min.	Mean.	Mean.			Max.	Min.	Mean.	Mean.	
	°C.	°C.	°C.	°C.	*Inches.*		°C.	°C.	°C.	°C.	*Inches.*
May 1	9.0	6.0	7.5	6.2	0.21	June 1	28.5	19.0	23.7	21.5	0.75
2	......	......	......	8.5	.0	2	27.5	18.5	23.0	20.8	.37
3	9.5	.7	8.2	7.0	.33	3	26.0	19.0	22.5	20.3	Trace.
4	14.8	7.0	10.9	8.7	.21	4	22.0	14.0	18.0	15.8	.39
5	13.8	7.5	10.6	8.5	.0	5	23.0	14.0	18.5	16.3	.01
6	14.8	7.0	10.9	8.0	.63	6	24.1	14.0	19.0	16.8	.04
7	21.2	7.5	14.4	10.9	.0	7	25.5	13.0	19.2	17.0	.01
8	16.2	8.0	11.1	9.4	.0	8	28.0	11.5	20.2	18.0	.0
9	20.0	5.0	12.5	9.2	.0	9	28.2	20.5	24.3	22.1	.18
10	20.9	6.0	13.4	9.8	.0	10	29.8	18.0	23.9	21.7	.18
11	23.6	6.5	16.5	11.1	.0	11	29.5	17.6	23.5	21.3	.74
12	22.9	8.0	15.4	12.6	.0	12	29.0	19.0	24.0	21.8	.0
13	22.8	6.8	14.8	13.9	.0	13	31.0	20.0	25.5	23.3	.02
14	22.5	7.5	15.0	13.5	Trace.	14	30.5	20.5	25.5	23.3	.6
15	17.2	7.8	12.5	10.3	.03	15	21.8	21.0	26.4	24.2	.0
16	19.5	9.0	14.2	12.0	.09	16	30.0	22.0	26.0	23.8	Trace.
17	22.5	7.5	15.0	14.9	.0	17	31.2	22.0	26.6	24.4	.0
18	24.5	7.0	15.7	14.5	.0	18	31.8	21.0	26.4	24.2	.0
19	20.0	10.5	15.2	13.5	.0	19	34.5	21.6	26.0	23.8	.02
20	13.8	7.0	10.4	8.2	Trace.	20	33.5	22.5	28.3	26.1	Trace.
21	......	......	......	......	.2	21	32.5	21.1	26.8	24.6	Trace.
22	......	......	......	......	.01	22	29.1	21.0	25.0	22.8	Trace.
23	17.5	10.5	14.0	11.8	.01	23	27.0	21.8	24.4	22.2	Trace.
24	23.2	6.5	14.8	12.6	.0	24	28.8	21.5	25.1	22.9	.05
25	27.8	9.5	18.6	16.4	.0	25	31.8	21.0	26.0	23.8	Trace.
26	27.8	11.4	12.6	10.4	.0	26	27.5	21.8	29.6	27.4	.0
27	30.0	13.0	21.5	19.3	.0	27	23.8	19.5	21.6	19.4	.0
28	30.5	14.2	22.3	20.1	.0	28	30.5	15.2	22.8	20.6	.0
29	32.5	14.0	23.2	21.0	.0	29	32.8	16.0	24.4	22.2	.0
30	32.8	15.0	23.9	21.7	.02	30	3.4	17.5	25.7	25.7	.0
31	32.2	18.0	25.1	22.9	1.72						

[1] The data on the daily rainfall here presented were obtained from the monthly meteorological summary for May and June, 1919, published by the local office of the U. S. Weather Bureau, Milwaukee,, Wis., v. 24, p. 36-37 and 44-45.

For the entire period from April 26 to May 25 an average daily mean temperature of 13.5° C. was maintained at a depth of 2 inches. Fortunately for the purpose of the experiment, between May 25 and 28 the weather turned suddenly warmer and the soil temperature at once arose to an average daily mean of 23.5° (fig. 1). This high daily mean was maintained thereafter until the conclusion of the experiment.

As is evident from the temperature data submitted in Table II, the stems grown from the tubers planted April 28 to May 7 grew through cold soil at temperatures ranging on the average well below 21° C.—the upper temperature limit shown in the greenhouse experiments at which the fungus may attack and destroy the young growing tips. The low temperatures which were maintained up to May 24 so retarded the growth of the young shoots as to afford opportunity for the accumulation of the fungus in the sinus of the young buds, resulting finally in the serious destruction of the growing points noted in the two earlier crops. In view of the very low temperatures maintained during the early part of May it is interesting to recall that growing-point destruction was

found under certain conditions in the greenhouse experiments to reach its maximum expression at temperatures as low as 12°.

In the case of the two later plantings the young potato shoots pushed through the soil at a daily mean temperature approximating very closely the optimum for this early period of growth (24° C.) at which temperature the pathogenicity of the fungus, as previously reported, is definitely inhibited. This increased rate of growth of the host, together with the lessened parasitic action of the fungus at the higher soil temperatures, accounts, in a large measure, for the few growing points injured and for the decreased severity of the cortical lesions noted in the later crops.

The sudden rise in the soil temperature might account in a similar way for the absence of injury to the primordia of the secondary shoots

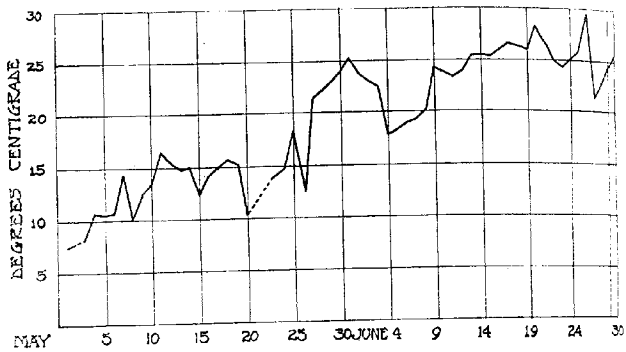

Fig. 1.—Graph showing the range of the mean daily soil temperature at a depth of 2 inches for Madison, Wis., during the months of May and June, 1919. An average mean of 13.5° C. was maintained from April 26 to May 25. Between May 25 and 28 the temperature of the soil increased to a daily mean of 23.5° for the period of May 28 to June 30 (Table II and the discussion, p. 763).

noted in the later two crops (Pl. 1, A, B). The primary stems from these early plantings, as suggested, grew in cold soil which so greatly retarded their rate of growth as to permit girdling and killing of the tender buds. Later with the rise in the temperature, the secondary shoots arising from these injured primary sprouts were permitted to grow so rapidly through the soil as to escape practically uninjured. This is especially significant in view of the fact that the fungus undoubtedly was present in the soil in greater abundance at this later date.

EVIDENCE OF THE IMPORTANCE OF SOIL TEMPERATURE FROM SEED TREATMENT AND PATHOGENICITY TESTS AT PLAINFIELD, WIS.

During 1918 field studies were made at Plainfield, Wis., for the purpose of determining the relation of *Corticium vagum* to seed-potato treatment and to crop development. These studies were repeated at the same place under comparable conditions in 1919.[6] Such uniformity of cultural methods and general field technic were employed for the two seasons as to control as far as possible under field conditions the various factors which might affect the virulence of the fungus. No attempt, however, was made to modify either the soil temperature or the soil moisture content.

[6] The details of these field tests will be published at a later date; only such data as relate definitely to the effects of soil temperature upon the pathogenicity of *Corticium vagum* are included in this paper.

The plots used for the experiments in the two different years were separated by a distance of but a few rods; both were equally level, and the soil was uniform in structure and texture. Seed for the two seasons was obtained from the same stock which had been grown under comparable conditions for a period of seven years on the farm on which the experiments were located. All tubers used for seed were selected with equal care each season for the uniform presence of the sclerotia of *Corticium vagum*. These were cut in halves and planted 5 inches deep at 12-inch intervals in parallel rows 3½ feet apart. The same method of taking observations was employed for the two seasons.

In 1918, 22 rows were planted with 100 hills in each. The seed for 17 of these rows was treated variously with mercuric-chlorid and formaldehyde solution. The remaining 5 rows, placed at regular intervals throughout the plot, were planted with untreated seed for controls. Plantings were made on June 13 and 14.

The first data from the 1918 experiments were taken on July 19, five weeks after planting. At this date a very striking difference appeared between the control rows and the rows grown from treated seed (Pl. 1, C). The plants in the control rows were definitely undersized and irregular; "misses" were frequent, and many of the plants were just breaking soil, while plants in adjacent rows from treated seed were 10 inches or more in height (Pl. 1, C). The examination of alternate plants in the various rows at this time showed severe stem lesions on 80 per cent of the plants from the untreated seed. Numerous primary, secondary, and even tertiary growing tips were destroyed, with total killing of the stems in many instances. The destructive attack on the secondary and tertiary stems is shown in the loss of 16 per cent of the stand and further by the fact that the number of stems per hill from the untreated seed was reduced over 26 per cent as compared with the number from treated seed.

The final results obtained October 12 are equally definite in showing the severity of the disease during the season. Compared with the healthy plants the hills from untreated seed showed an average decrease in the number of tubers produced of 20.7 per cent with a corresponding 34.2 per cent decrease in weight per hill. This average decrease in weight per hill, together with the 16 per cent loss in the stand, resulted in an aggregate loss to the crop from the untreated tubers of approximately 50 per cent.

Table III.—*Comparative summary of the results obtained from the Plainfield, Wis., plots during the seasons 1918 and 1919* [1]

Year.	Number of rows.	Number of hills.	Number of control rows.	Number of control hills.	Average hills showing lesions.	Average decrease in stems per hill.	Average decrease in stand.	Average decrease in yield per hill.	Average decrease in tubers per hill.	Average loss to crop.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1918	22	2,200	5	500	80.0	26.1	16.0	34.2	20.7	50.0
1919	35	3,500	7	700	75.1	Slight Increase.	2.8	12.2	14.3	15.4

[1] The figures in the table are obtained by comparing the plants grown from sclerotia-covered untreated seed with those growing from the same seed equally covered with sclerotia but treated variously with mercuric chlorid.

TABLE IV.—*Daily mean air and soil temperatures and daily rainfall for Hancock,[1] Wis., June, 1918 and 1919*

Date.	Daily mean air temperature for Hancock.[2]		Daily mean soil temperature for Plainfield.		Daily rainfall for Hancock.	
	1918	1919	1918[3]	1919	1918	1919
	°C.	°C.	°C.	°C.	Inches.	Inches.
May 26	17.5	20.8	18.5	....	....	....
27	18.0	20.8	19.0	20.6	....	....
28	12.2	20.8	13.2	22.0	....	....
29	8.8	20.8	9.8	22.5	....	....
30	17.2	24.4	18.2	23.1	....	....
31	25.0	25.8	26.0	24.5	....	....
June 1	21.9	22.7	22.9	23.2	0.30	1.44
2	19.7	21.9	20.7	21.4	....	1.09
3	17.2	19.1	18.2	19.5	....	....
4	16.9	20.8	17.9	19.9	Trace.	.09
5	19.5	18.1	20.5	17.9	.05	....
6	19.4	15.8	20.4	16.5	Trace.	.09
7	15.0	17.5	16.0	18.2	....	.43
8	16.1	16.1	17.1	17.3	.04	Trace.
9	18.6	20.3	19.6	20.8	.75	.01
10	17.2	23.1	18.2	21.9	....	.91
11	23.9	22.8	24.9	21.5	....	.04
12	19.4	24.7	20.4	23.5	....	....
13	17.7	25.3	18.7	24.2	....	.34
14	19.7	24.7	20.7	25.7	....	....
15	17.2	25.3	18.2	26.1	....	....
16	26.7	23.9	27.7	27.5	....	.18
17	23.1	24.4	24.1	24.6	....	.02
18	16.7	25.5	17.9	26.0	.09	....
19	19.7	26.1	20.7	26.1	....	....
20	18.1	23.3	19.1	23.8	Trace.	....
21	18.3	....	19.3	24.3	Trace.	....
22	14.4	22.2	15.4	23.7	....	....
23	16.1	22.8	17.1	22.5	....	....
24	22.2	23.6	23.2	22.8	....	1.00
25	18.5	24.7	19.5	23.1	....	.05
26	20.3	25.0	21.3	25.0	....	....
27	20.3	20.0	21.3	21.5	....	....
28	21.1	14.4	22.1	20.7	Trace.	....
29	19.7	18.3	20.7	22.0	Trace.	....
30	18.6	22.8	18.6	....	1.26	....
Mean average	19.1	22.0	20.1	23.0	....	....
Total rainfall for June	....	....	....	....	2.49	5.6[illegible]

[1] The soil temperatures were determined and calculated for a depth of 4 inches.
The nearest weather recording station to Plainfield was at Hancock, Wis., 6 miles southwest. The soil and weather conditions at the two places are in general very similar. The average daily mean soil temperature at a depth of 4 inches at Plainfield during 1919 was found to be just 1° C. higher than the average daily mean air temperature recorded for the same day at Hancock. Assuming the same relation to hold true in 1918, a fair index is obtained for calculating both the soil and the air temperature at Plainfield for the season of 1918.

[2] The daily air temperatures for Hancock for May and June, 1918 and 1919, were calculated directly from the Wisconsin section of the Climatological Data, Weather Bureau, United States Department of Agriculture.

[3] Calculated.

In 1919, 35 parallel rows each containing 100 hills were planted as in 1918. Seven of these rows were planted with untreated seed as controls. Plantings were made May 25 and the first observations on July 3, five weeks later. At this later date the ravages of the disease were not nearly so apparent as in 1918. While the control rows could be detected

with little difficulty by the irregular size of the plants, yet "misses" were infrequent and many of the control plants were equal in size to those from treated seed. Fewer growing points were destroyed than was noted in 1918, while few, if any, secondary stems were seriously attacked. The lesions on the whole were much less severe than those of the previous year, as is shown by the fact that no reduction occurred either in the stand or in the number of stems per hill in the diseased as compared with the healthy hills. The relative severity of the disease is further shown by the smaller decrease in the yield from the diseased hills, as indicated both in the weight and number of tubers per hill. In every respect the action of the fungus was definitely less severe during 1919 than during 1918. The comparative results are shown in Table III.

As stated, the most important uncontrolled factors operating during the two seasons of 1918 and 1919 were soil temperature and soil moisture. The precipitation was much lighter during the first four weeks after planting in 1918 than during the same period of 1919, as is shown in Table IV. As shown in Table V, during June, 1918, 2.49 inches fell, as compared with 5.69 inches during June, 1919. For the second, third, and fourth weeks of growth in 1918 and 1919 the rainfall was 2.24 and 4.23 inches, respectively. It should be noted, however, that during the month of May, 1918, 9.78 inches fell, while 4.08 inches of rain fell in May, 1919. This early rainfall no doubt influences greatly the amount of moisture in the soil during this early part of the crop development.

TABLE V.—*Monthly mean of daily temperature and total monthly rainfall for Hancock, Wis., during the potato-growing season of 1918 and 1919*

Month.	Average daily mean air temperature.		Total rainfall.	
	1918	1919	1918	1919
Iay	16.0	13.1	9.78	4.08
une	19.1	22.0	2.49	5.69
uly	21.25	23.2	1.92	2.13
ugust	22.7	19.0	3.12	2.31
eptember	12.75	....	1.31	....

By far the greatest damage to the crop occurred during the season of east rainfall. While little positive evidence is available as to the exact ffect of moisture on the pathogenic action of *Corticium vagum* on the otato, it has been generally assumed to be more virulent in relatively amp soil. It is hardly probable that the decreased rainfall during 1918 unctioned as an important factor in the greater damage done by the ungus during that year. It is suggested in this connection that the low oisture content of the soil during 1918 might have acted as a retarding ctor in the growth of the young shoot through the soils, thus contributg to the severity of the disease. No indication of such retardation, owever, was noted. In fact, the crop on the plots in general exhibited reater vitality during the early season of 1918 than during the same eriod of 1919.

In view of the known facts the relation of soil temperature to the variaion in the severity of the disease during 1918 and 1919 appears more clear.

Fortunately, again, for the purpose of the experiments the temperature for the two years differed widely. June, 1918, was cool, averaging 0.11° C. below normal for the locality, while June, 1919, was the warmest that had been experienced in Hancock and surrounding districts for a long period of years, with an average of 2.6° above normal. This high average, it is evident from the figures submitted in Table IV, was due to uniformly high temperatures rather than to any period of excessively high temperatures. Mr. H. B. Hersey, Director of the Wisconsin Section of the U. S. Weather Bureau, states that—

> June was unusually warm. The average temperature for the State, 69.4° F., has been exceeded only once in June since records began in 1891.

The actual daily mean air temperature for Hancock for the month of June was 19.1° C. in 1918 and 22° for the same month in 1919. The average mean daily soil temperature of 23° was determined for Plainfield during June in 1919 with a calculated soil temperature of 20.1° for 1918 (Table IV), or a difference of 2.9°. For the second, third, and fourth weeks after planting the average daily mean temperature calculated for 1918 was 19.4° with an actual daily mean of 22.8° for 1919, a difference of 2.4°.

These temperature relations are especially significant when considered in view of the critical temperature for growing-point injury determined in the more carefully controlled greenhouse experiments. As previously indicated, no damage was found to occur to the growing points of the young potato shoots above 21° C., while below this to as low as 12° serious destruction of the young bud resulted. In 1918 the average mean soil temperature during the critical period of growth the shoots through the soil remained below this critical temperature, while in 1919 the average daily mean was well above 21°. Growing-point destruction was almost wholly responsible for the reduced stand and the decreased number of stems per hill in the control rows in the 1918 crop. It is also significant that the average daily mean of 20.1° maintained during June of 1918 approaches closely the soil temperature (18°) found optimum for the destruction of cortical tissue of the young stems, while during June, 1919, which included the entire time which the young shoots were growing through the soil, the average daily mean soil temperature approximated closely that temperature (24°) found in the greenhouse experiments to be optimum for the early growth of the young potato shoots and at which temperature the fungus, due to some inhibitive factor, ceases to be seriously parasitic on potato stems. In view of the facts, it appears evident that the greater damage to the 1918 crop, amounting to three times that of 1919, was occasioned primarily by low soil temperature during that season. The high soil temperature, on the other hand, appears equally responsible for the inhibited action of the pathogen during 1919.

DISCUSSION

Results obtained from both the field and greenhouse experiments emphasize clearly the controlling influence of soil temperature upon the pathogenic power of *Corticium vagum*. While the fungus, as was shown in the earlier studies, may produce lesions on the underground parts of the potato over a relatively wide range of soil temperature, it has become evident that variations in the average mean soil temperature of 2° or 3° above or below 21° C. and maintained for the first few weeks after planting may determine accurately the degree to which this fungus may

damage the potato crop. However, from the standpoint of practical methods of control, except for the possible advantage gained by late planting, we may expect but little from temperature relation of the fungus. This is the more evident considering the fact, as pointed out in the earlier publications, that the fungus operates most vigorously as a parasite under such temperature as best favors maximum potato production.

The generally accepted idea borne out by these field experiments that the early crop of potatoes is more severely damaged by *Corticium vagum* appears to be largely a question of the cooler soil temperatures maintained during the early growth of the plants. What part may be played by the higher soil moisture content generally occurring during these earlier dates remains undetermined. Whether such increased soil moisture, if important, contributes directly to the pathogenic activity of the fungus or only indirectly by its influence on the temperature of the soil is not clear. From the greenhouse experiments in which the moisture factor was carefully controlled, it would appear evident that such variations as frequently occur in the temperature values between early and late planting might adequately account for such differences as are noted in the pathogenicity of the fungus in question. Again, the greater susceptibility to the attack of *C. vagum* frequently attributed to our standard early varieties enters in as a possible factor in the greater damage to the early crops. Here again it is not improbable that the suggested susceptibility is induced by the particular environmental conditions to which these early varieties are exposed. The possible increased virulence of the fungus produced by a cooler soil evidently functions here as an important factor.

Such determination of the various temperature relations of *Corticium vagum* made during these studies may add much by way of explanation of many of the conflicting opinions held regarding the pathogenicity of the fungus as a potato parasite. Seasonal variation in the severity of the stem canker is easily explained in view of the facts presented. Special significance is also given to the observation that the stem canker caused by *C. vagum* reaches its maximum severity in the northern potato sections of Canada and the States of Maine, Wisconsin, and Michigan, as also in the higher altitudes of the Western States. Clearly, however, the fungus may become especially virulent on the very early crops even in the warmer areas.

SUMMARY

(1) Special significance is given to the soil temperature relations of *Corticium vagum* established in the earlier greenhouse experiments by the field studies conducted during 1918 and 1919. These studies further emphasize the importance of the soil temperature as a controlling factor in the pathogenicity of the sterile stage of *C. vagum* on the potato.

(2) *Corticium vagum* caused greater growing-point destruction and a more severe type of cortical injury on the early potato crop planted April 26 and May 7 than on the later plantings of May 17 and 30. This difference was due to a sudden rise in the soil temperature at such dates as to permit early growth of the later crops to take place in a very warm soil.

(3) Growing tips of the young primary shoots grown from potato tubers planted at the early dates of April 26 and May 7 were destroyed in cold soil by *Corticium vagum*, while secondary primordia developed from such injured shoots later grew through warm soil uninjured.

(4) Greater damage to potato plants occurred in the field in sclerotia-inoculated soil during the cool season of 1918 with a daily mean soil temperature for June of 20.1° C. than during 1919 with an average daily mean of 23° for the same growth period. This loss in 1918 was expressed in a greater decreased stand, a greater number and a more severe type of stem lesions, fewer stems per hill, decreased number of tubers per hill, and a total loss in yield of 50 per cent as opposed to a total loss of 15.4 per cent in 1919.

(5) The results in general indicate that the occurrence of an average daily mean temperature of 2° above or below the critical temperature (21°) for the pathogenicity of the *Corticium vagum* on the growing points during the first six weeks of potato growth may accurately govern the damage done to the crop by this fungus in any one season.

(6) Such temperature data as are here presented will go far in helping to explain many of the conflicting views held regarding the pathogenicity of *Corticium vagum* on the potato and other hosts.

PLATE I

A.—Irish Cobbler potatoes grown under field conditions in soil inoculated with a pure culture of *Corticium vagum*. Plant grown from tuber planted April 26, showing the destruction of the primary growing point and uninjured secondary stem. Twenty-five per cent of the growing points of the primary shoots of the crop planted on this date were destroyed (Table I).

B.—Same as A except that plant was grown from tuber planted May 7. A total of 25.5 per cent of the primary buds of this crop were destroyed.

The photographs illustrate how the growing tips of the primary shoots growing at the lower temperatures were attacked and killed by the fungus. Later secondary shoots arose from the primary stems which with the advent of higher soil temperatures grew so rapidly through the soil as to escape uninjured. The decreased pathogenic power of the fungus at the higher temperature no doubt aided in this escape.

C.—Potato plot at Plainfield, Wis., showing the disastrous effects of *Corticium vagum* on the potato crop during the cool season, 1918. The third row from the right was planted with sclerotia-covered, untreated tubers. The plants in the rows on either side were grown from tubers equally "scurfed" but treated with mercuric chlorid.